

MOUSE SKELETAL MUSCLE TROPONIN-I ELISA KIT

Life Diagnostics, Inc., Cat. No. 2020-1-SK

MOUSE SKELETAL MUSCLE TROPONIN-I (SkM-TnI) ELISA

STORAGE CONDITIONS

- Store the SkM-TnI Stock vials at or below -20°C
- Store the remainder of the kit at 2-8°C
- Keep the microtiter plate in a sealed bag with desiccant to minimize exposure to damp air

EXPIRATION

The kit expiration date is indicated on the package.

BACKGROUND

Troponin is the contractile regulating protein complex of striated muscle. It consists of three distinct polypeptides: troponin-I, troponin-C, and troponin-T. The troponin-I subunit exists in three distinct isoforms; one each in fast-twitch and slow-twitch skeletal muscle fibers, and one in cardiac muscle. Following muscle injury, troponin-I is released into the blood and measurement of troponin-I in serum or plasma provides a measurement of the extent of muscle injury. This ELISA kit uses a detection antibody that is specific for the fast twitch isoform of troponin-I, thereby allowing specific evaluation of skeletal muscle injury.

PRINCIPLE OF THE ASSAY

The assay uses two different antibodies. A polyclonal antibody specific for skeletal muscle troponin-I is used for solid phase immobilization (on the microtiter wells). A monoclonal antibody specific for fast twitch skeletal muscle troponin-I and conjugated to horse radish peroxidase (HRP) is used for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes after which the wells are washed and HRP conjugate is added and incubated for 45 minutes. This results in troponin-I molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent (HRP substrate solution) is added and incubated for 20 minutes. If troponin-I is present a blue color develops. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of troponin-I is proportional to the optical density of the test sample.

REAGENTS AND MATERIALS PROVIDED

- Anti SkM-TnI Coated Wells (1 plate, 96 wells)
- SkM-TnI Stock (3 vials): Lyophilized mouse SkM-TnI (reconstitute with 0.10 ml H₂O)
- Standard Diluent (50 ml)
- Sample Diluent (25 ml)
- Wash Buffer (20x stock, 50 ml)
- Anti SkM-TnI HRP Conjugate (11 ml)
- TMB Reagent (11 ml)
- Stop Solution (11 ml): 1N HCl

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Pipettes: P-10, P-200 & P-1000 or equivalent
- Disposable pipette tips
- Microtiter well reader capable of reading OD at 450 nm
- Vortex mixer
- Absorbent paper
- Graph paper or appropriate PC graphing software
- Polypropylene microcentrifuge tubes (1.5 ml)

WARNINGS AND PRECAUTIONS

- Avoid contact with 1N HCl (Stop Solution). It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
- Do not use reagents after expiration date and do not mix or use components from different kits.
- Replace caps on reagents immediately. Do not switch caps.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Equilibrate kit components to room temperature before use.
2. Reconstitute one vial of the lyophilized SkM-TnI stock by addition of 100 µl of de-ionized or distilled water. Mix gently until dissolved – **USE WITHIN 30 MINUTES OF RECONSTITUTION**. The concentration of SkM-TnI in the reconstituted stock is indicated on the vial label.
3. Label 7 polypropylene tubes as 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0 ng/ml.
4. Into the tube labeled 50 ng/ml, pipette the volume of **Standard Diluent** detailed on the SkM-TnI stock vial label. Then add the indicated volume of SkM-TnI stock (shown on the SkM-TnI stock vial label) and mix gently. This provides the 50 ng/ml standard.
5. Pipette 0.25 ml of **Standard Diluent** into the tubes labeled 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0 ng/ml
6. Prepare a 25 ng/ml standard by diluting and mixing 0.25 ml of the 50 ng/ml standard with 0.25 ml of standard diluent in the tube labeled 25 ng/ml. Similarly prepare the 12.5, 6.25, 3.125, 1.56 and 0.78 ng/ml standards by serial dilution.

NOTE: The reconstituted SkM-TnI standards should be used within 30 minutes of stock reconstitution. Discard the stock after use.

SAMPLE COLLECTION

Serum or plasma (EDTA) should be prepared as quickly as possible after blood collection and stored at 4°C. All samples should be similarly processed (i.e., storage times and temperatures should be the same for all samples). If samples cannot be assayed

within 1-2 hours of collection they should be frozen at -70°C and thawed only once prior to use.

SAMPLE PREPARATION

In studies at Life Diagnostics, Inc., we have encountered samples with very low (≤ 4 ng/ml) and high (> 500 ng/ml) levels of troponin-I. Depending on the level of troponin-I two different methods of sample preparation are recommended.

1. Low troponin-I levels: plasma or serum samples should be diluted with 1/3rd volume of **Sample Diluent** (i.e., 180 μl of serum or plasma should be diluted with 60 μl of sample diluent).
2. High troponin-I levels: If samples prepared as described in 1 above give absorbance values that exceed those of the 100 ng/ml standard, samples pre-diluted with Sample diluent as described above should be further diluted with **Standard diluent** (i.e., one volume of sample pre-diluted as described in 1 above, should be mixed directly with one or more volumes of Standard diluent).

We recommend that samples be assayed in duplicate. Wherever possible, all samples should be similarly diluted in order to avoid minor matrix differences.

PROCEDURAL NOTES

1. Standards and diluted samples should be prepared immediately prior to use and used within 30 minutes.
2. Pipetting of all standards, samples and conjugate into the microtiter plate should be completed within 10 minutes.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μl of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature ($18-25^{\circ}\text{C}$) for 45 minutes.
4. Remove the incubation mixture using either a plate washer or by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 5 times with 1x wash solution using a plate washer (400 μl /well). The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Add 100 μl of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature ($18-25^{\circ}\text{C}$) for 45 minutes.
9. Wash as detailed in 4 to 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
11. Dispense 100 μl of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature ($18-25^{\circ}\text{C}$) for 20 minutes.
13. Stop the reaction by adding 100 μl of Stop Solution to each well.
14. Gently mix. *It is important to make sure that all the blue color changes to yellow.*

15. Read the optical density at 450 nm with a microtiter plate reader *within 5 minutes*.
16. If absorbance values of samples exceed that of the 50 ng/ml standard, samples should be appropriately diluted and re-tested.

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for the standards and samples.
2. Construct a standard curve by plotting the A_{450} values obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the A_{450} values for each sample, determine the corresponding concentration of SkM-TnI (ng/ml) from the standard curve. If using graphing software, we suggest using a linear regression fit of the data.
5. Multiply the derived SkM-TnI concentrations by the dilution factor (i.e., 1.33, if the "low troponin-I level" dilution procedure was used) to obtain the actual SkM-TnI concentration.

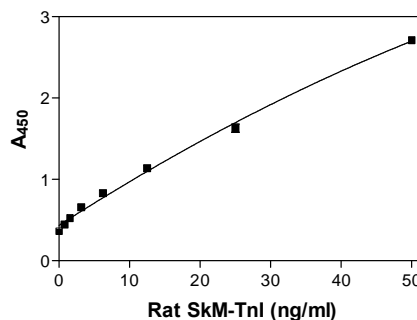
EXAMPLE OF STANDARD CURVE

Results of a typical standard curve with A_{450} plotted on the Y axis against cTnI concentrations on the X axis are shown below.

NOTE: This standard curve is for the purpose of illustration only.

SkM-TnI (ng/ml)	Absorbance (450 nm)
50	2.713
25	1.629
12.5	1.142
6.25	0.830
3.125	0.658
1.56	0.527
0.78	0.448
0	0.363

Typical Standard Curve



LIMITATIONS OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

MOUSE MYOGLOBIN ELISA

Life Diagnostics, Inc., Catalog Number: 2110-1-N

ELISA for the Determination of Myoglobin in Mouse Serum, Plasma & Urine¹

STORAGE

Store standard at -20°C
STORE REMAINDER OF KIT AT 2 - 8°C

INTRODUCTION

Myoglobin is a heme protein found in both cardiac and skeletal muscle. Following myocardial necrosis associated with myocardial infarction (MI), myoglobin is one of the first markers to rise above normal levels. Studies with human subjects have shown that myoglobin increases measurably above baseline within 2-4 hours post-infarct, peaking at 9-12 hours, and returning to baseline within 24-36 hours. In the absence of skeletal muscle trauma or other factors associated with a noncardiac related increase in circulating myoglobin, myoglobin may be used as a marker for MI. Similarly, in the absence of cardiac damage, myoglobin may be used as a marker of skeletal muscle injury.

PRINCIPLE OF THE TEST

The Myoglobin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. A monoclonal anti-myoglobin antibody is used for solid phase immobilization (on the microtiter wells) and a polyclonal anti-myoglobin antibody conjugated to horseradish peroxidase is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the myoglobin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After one hour incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A TMB (Tetramethyl-benzidine) Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of myoglobin is proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- Anti-Myoglobin-coated microtiter wells, 96 wells
- Mouse Myoglobin Stock (50 µl, 50 µg/ml) **Store at -20°C**
- Diluent, 12 ml
- Enzyme Conjugate Reagent, 11 ml
- 20x Wash Solution, 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

¹ A matrix effect may be observed with urine samples that results in slight differences in absorbance values relative to myoglobin diluted in the ELISA diluent. It is therefore recommended that wherever possible all urine samples within a particular study be similarly diluted prior to testing, thereby ensuring an accurate determination of relative myoglobin levels within the study.

Materials required but not provided:

- Precision pipettes
- Disposable pipette tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Graph paper (PC graphing software is optional but recommended)
- Plate shaker
- Microtiter plate reader

INSTRUMENTATION

A microtiter plate reader with an optical density range of 0-4 OD at 450 nm wavelength is required.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Label 8 polypropylene tubes as 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 0 ng/ml.
2. Pipette 398 µl of diluent into the tube labeled 250 ng/ml
3. Pipette 100 µl of diluent into the tubes labeled 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 0 ng/ml.
4. Briefly centrifuge or flick the myoglobin stock tube to ensure that the liquid contents are at the bottom of the tube.
5. Dilute 2 µl of the 50 µg/ml myoglobin stock into the 398 µl of diluent in the tube labeled 250 ng/ml. This provides a 250 ng/ml solution of myoglobin.
6. Prepare a 125 ng/ml stock by diluting and mixing 100 µl of the 250 ng/ml stock with 100 µl of diluent in the tube labeled 125 ng/ml. Similarly prepare the 62.5, 31.25, 15.63, 7.81 and 3.91 ng/ml stocks by serial dilution.

SAMPLE COLLECTION

Serum, plasma and urine should be collected using standard techniques. Remove serum or plasma from the coagulated or packed cells within 60 minutes after collection. Plasma samples may be collected into tubes containing EDTA. Samples that cannot be assayed within 3 hours of collection should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed prior to testing.

SAMPLE PREPARATION

Samples may be tested undiluted or after dilution with diluent. The dilution factor should be determined empirically. On occasion a matrix effect may be observed with urine samples that may slightly increase or decrease absorbance values and we therefore strongly recommend that all urine samples within a particular study be similarly diluted. *Only 20 µl of*

sample is required per assay (2 x 20 µl, if samples are to be tested in duplicate).

PROCEDURAL NOTES

- Standards should be prepared immediately prior to use and should be used within 30 minutes of preparation.
- Pipetting of conjugate, standards and samples into the microtiter plate should be completed within 10 minutes.
- We recommend that standards and samples be run in duplicate.
- It is recommended that the wells be read within 5 minutes following addition of Stop Solution.

ASSAY PROCEDURE

- Ensure that all reagents are at room temperature.
- Secure the desired number of coated wells in the holder.
- Dispense 100 µl of Enzyme Conjugate Reagent into each well.
- Dispense 20 µl of myoglobin standards and samples (in duplicate) into the appropriate wells.
- Incubate at room temperature (18-25°C) on a plate shaker for one hour. Mix Gently (~100-150 rpm)
- Remove the incubation mixture either with a plate washer or by flicking plate contents into a waste container.
- Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 µl/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
- Dispense 100 µl of TMB Reagent solution into each well. Gently mix for 5 seconds.
- Incubate on a plate shaker at room temperature for 20 minutes. Mix Gently
- Stop the reaction by adding 100 µl of Stop Solution to each well.
- Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
- Read absorbance at 450 nm with a microtiter well reader within 5 minutes.

CALCULATION OF RESULTS

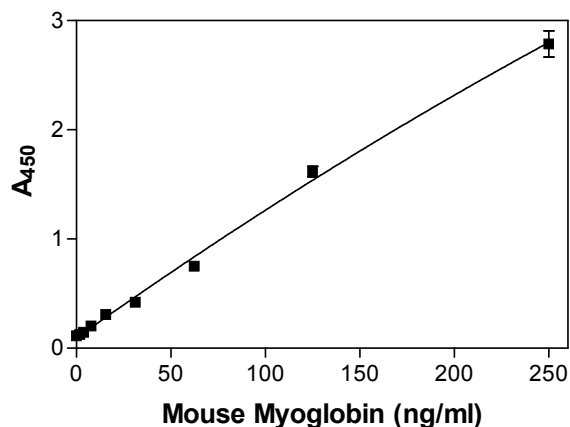
- Calculate the mean absorbance value for each set of reference standards and samples.
- Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
- Use the mean absorbance values for each sample to determine the corresponding concentration of myoglobin in ng/ml from the standard curve.
- Multiply the derived value by the appropriate dilution factor if the test samples were diluted.
- Graphing software, if available, should be used.

TYPICAL STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against myoglobin concentrations shown in the X axis are illustrated below. This standard curve is for illustrative purposes only, and should not be used to calculate unknowns. A standard curve should be run for each assay.

Myoglobin (ng/ml)	Absorbance (450 nm)
250	2.754
125	1.571
62.5	0.722
31.25	0.412
15.63	0.301
7.81	0.203
3.91	0.147
0	0.114

Typical Mouse Myoglobin Standard Curve



LIMITATIONS OF THE PROCEDURE

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

MONKEY SKELETAL MUSCLE TROPONIN-I ELISA KIT

Life Diagnostics, Inc., Cat. No. 2020-3-SK

MONKEY SKELETAL MUSCLE TROPONIN-I (SkM-TnI) ELISA

STORAGE CONDITIONS

- Store the SkM-TnI Stock vials at or below -20°C
- Store the remainder of the kit at 2-8°C
- Keep the microtiter plate in a sealed bag with desiccant to minimize exposure to damp air

EXPIRATION

The kit expiration date is indicated on the package.

BACKGROUND

Troponin is the contractile regulating protein complex of striated muscle. It consists of three distinct polypeptides: troponin-I, troponin-C, and troponin-T. The troponin-I subunit exists in three distinct isoforms; one each in fast-twitch and slow-twitch skeletal muscle fibers, and one in cardiac muscle. Following muscle injury, troponin-I is released into the blood and measurement of troponin-I in serum or plasma provides a measurement of the extent of muscle injury. This ELISA kit uses a detection antibody that is specific for the fast twitch isoform of troponin-I, thereby allowing specific evaluation of skeletal muscle injury.

PRINCIPLE OF THE ASSAY

The assay uses two different antibodies. A polyclonal antibody specific for skeletal muscle troponin-I is used for solid phase immobilization (on the microtiter wells). A monoclonal antibody specific for fast twitch skeletal muscle troponin-I and conjugated to horse radish peroxidase (HRP) is used for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes after which the wells are washed and HRP conjugate is added and incubated for 45 minutes. This results in troponin-I molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent (HRP substrate solution) is added and incubated for 20 minutes. If troponin-I is present a blue color develops. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of troponin-I is proportional to the optical density of the test sample.

REAGENTS AND MATERIALS PROVIDED

- Anti SkM-TnI Coated Wells (1 plate, 96 wells)
- SkM-TnI Stock¹ (3 vials): Lyophilized SkM-TnI (reconstitute with 0.10 ml H₂O)
- Standard Diluent (50 ml)
- Sample Diluent (25 ml)

- Wash Buffer (20x stock, 50 ml)
- Anti SkM-TnI HRP Conjugate (11 ml)
- TMB Reagent (11 ml)
- Stop Solution (11 ml): 1N HCl

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Pipettes: P-10, P-200 & P-1000 or equivalent
- Disposable pipette tips
- Microtiter well reader capable of reading OD at 450 nm
- Vortex mixer
- Absorbent paper
- Graph paper or appropriate PC graphing software
- Polypropylene microcentrifuge tubes (1.5 ml)

WARNINGS AND PRECAUTIONS

- Avoid contact with 1N HCl (Stop Solution). It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
- Do not use reagents after expiration date and do not mix or use components from different kits.
- Replace caps on reagents immediately. Do not switch caps.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Equilibrate kit components to room temperature before use.
2. Reconstitute one vial of the lyophilized SkM-TnI stock by addition of 100 µl of de-ionized or distilled water. Mix gently until dissolved – **USE WITHIN 30 MINUTES OF RECONSTITUTION**. The concentration of SkM-TnI in the reconstituted stock is indicated on the vial label.
3. Label 7 polypropylene tubes as 200, 100, 50, 25, 12.5, 6.25, and 3.125 ng/ml.
4. Into the tube labeled 200 ng/ml, pipette the volume of **Standard Diluent** detailed on the SkM-TnI stock vial label. Then add the indicated volume of SkM-TnI stock (shown on the SkM-TnI stock vial label) and mix gently. This provides the 200 ng/ml standard.
5. Pipette 0.25 ml of **Standard Diluent** into the tubes labeled 100, 50, 25, 12.5, 6.25, and 3.125 ng/ml
6. Prepare a 100 ng/ml standard by diluting and mixing 0.25 ml of the 200 ng/ml standard with 0.25 ml of standard diluent in the tube labeled 100 ng/ml. Similarly prepare the 50, 25, 12.5, 6.25, and 3.125 ng/ml standards by serial dilution.

NOTE: The reconstituted SkM-TnI standards should be used within 30 minutes of stock reconstitution. Discard the stock after use.

¹ International import/export restrictions apply to monkey derived products. In order to avoid such restrictions the SkM-TnI standard supplied with this kit is of non monkey origin. The standard curve obtained with this material is identical to that obtained with monkey SkM-TnI.

SAMPLE COLLECTION

Serum or plasma (EDTA) should be prepared as quickly as possible after blood collection and stored at 4°C. All samples should be similarly processed (i.e., storage times and temperatures should be the same for all samples). If samples cannot be assayed within 1-2 hours of collection they should be frozen at -70°C and thawed only once prior to use.

SAMPLE PREPARATION

In studies at Life Diagnostics, Inc., we have encountered samples with very low (≤ 4 ng/ml) and high (> 500 ng/ml) levels of troponin-I. Depending on the level of troponin-I two different methods of sample preparation are recommended.

1. Low troponin-I levels: plasma or serum samples should be diluted with 1/3rd volume of **Sample Diluent** (i.e., 180 μ l of serum or plasma should be diluted with 60 μ l of sample diluent).
2. High troponin-I levels: If samples prepared as described in 1 above give absorbance values that exceed those of the 100 ng/ml standard, samples pre-diluted with Sample diluent as described above should be further diluted with **Standard diluent** (i.e., one volume of sample pre-diluted as described in 1 above, should be mixed directly with one or more volumes of Standard diluent).

We recommend that samples be assayed in duplicate. Wherever possible, all samples should be similarly diluted in order to avoid minor matrix differences.

PROCEDURAL NOTES

1. Standards and diluted samples should be prepared immediately prior to use and used within 30 minutes.
2. Pipetting of all standards, samples and conjugate into the microtiter plate should be completed within 5 minutes.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μ l of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
4. Remove the incubation mixture using either a plate washer or by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 6 times with 1x wash solution using a plate washer (400 μ l/well). The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Add 100 μ l of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
9. Wash as detailed in 4 to 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
11. Dispense 100 μ l of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.

13. Stop the reaction by adding 100 μ l of Stop Solution to each well.
14. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
15. Read the optical density at 450 nm with a microtiter plate reader *within 5 minutes*. **Please Note: Due to plate reader differences, the high standard absorbance values may be out of range when read at 450 nm. If this occurs, absorbance values may be determined at 405 nm instead.**
16. If absorbance values of samples exceed that of the 200 ng/ml standard, samples should be appropriately diluted and re-tested.

CALCULATION OF RESULTS

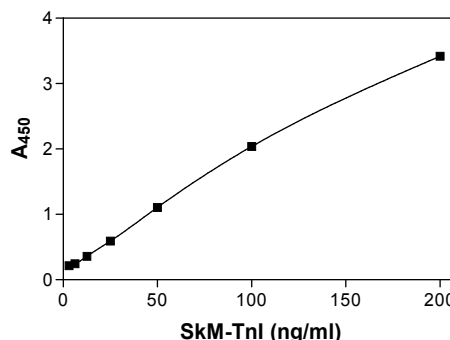
1. Calculate the mean absorbance value (A_{450}) for the standards and samples.
2. Construct a standard curve by plotting the A_{450} values obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the A_{450} values for each sample, determine the corresponding concentration of SkM-TnI (ng/ml) from the standard curve. If using graphing software, we suggest using a linear regression fit of the data.
5. Multiply the derived SkM-TnI concentrations by the dilution factor (i.e., 1.33, if the "low troponin-I level" dilution procedure was used) to obtain the actual SkM-TnI concentration.

EXAMPLE OF STANDARD CURVE

Results of a typical standard curve with A_{450} plotted on the Y axis against cTnI concentrations on the X axis are shown below. **NOTE:** This standard curve is for the purpose of illustration only.

SkM-TnI (ng/ml)	Absorbance (450 nm)
200	3.418
100	2.038
50	1.106
25	0.592
12.5	0.357
6.25	0.245
3.125	0.215

Representative Monkey SkM-TnI Standard Curve



LIMITATIONS OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

RAT SKELETAL MUSCLE TROPONIN-I ELISA KIT

Life Diagnostics, Inc., Cat. No. 2020-2-SK

RAT SKELETAL MUSCLE TROPONIN-I (SkM-TnI) ELISA

STORAGE CONDITIONS

- Store the SkM-TnI Stock vials at or below -20°C
- Store the remainder of the kit at 2-8°C
- Keep the microtiter plate in a sealed bag with desiccant to minimize exposure to damp air

EXPIRATION

The kit expiration date is indicated on the package.

BACKGROUND

Troponin is the contractile regulating protein complex of striated muscle. It consists of three distinct polypeptides: troponin-I, troponin-C, and troponin-T. The troponin-I subunit exists in three distinct isoforms; one each in fast-twitch and slow-twitch skeletal muscle fibers, and one in cardiac muscle. Following muscle injury, troponin-I is released into the blood and measurement of troponin-I in serum or plasma provides a measurement of the extent of muscle injury. This ELISA kit uses a detection antibody that is specific for the fast twitch isoform of troponin-I, thereby allowing specific evaluation of skeletal muscle injury.

PRINCIPLE OF THE ASSAY

The assay uses two different antibodies. A polyclonal antibody specific for skeletal muscle troponin-I is used for solid phase immobilization (on the microtiter wells). A monoclonal antibody specific for fast twitch skeletal muscle troponin-I and conjugated to horse radish peroxidase (HRP) is used for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes after which the wells are washed and HRP conjugate is added and incubated for 45 minutes. This results in troponin-I molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent (HRP substrate solution) is added and incubated for 20 minutes. If troponin-I is present a blue color develops. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of troponin-I is proportional to the optical density of the test sample.

REAGENTS AND MATERIALS PROVIDED

- Anti SkM-TnI Coated Wells (1 plate, 96 wells)
- SkM-TnI Stock (3 vials): Lyophilized rat SkM-TnI (reconstitute with 0.10 ml H₂O)
- Standard Diluent (50 ml)
- Sample Diluent (25 ml)
- Wash Buffer (20x stock, 50 ml)
- Anti SkM-TnI HRP Conjugate (11 ml)
- TMB Reagent (11 ml)
- Stop Solution (11 ml): 1N HCl

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Pipettes: P-10, P-200 & P-1000 or equivalent
- Disposable pipette tips
- Microtiter well reader capable of reading OD at 450 nm
- Vortex mixer
- Absorbent paper
- Graph paper or appropriate PC graphing software
- Polypropylene microcentrifuge tubes (1.5 ml)

WARNINGS AND PRECAUTIONS

- Avoid contact with 1N HCl (Stop Solution). It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
- Do not use reagents after expiration date and do not mix or use components from different kits.
- Replace caps on reagents immediately. Do not switch caps.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Equilibrate kit components to room temperature before use.
2. Reconstitute one vial of the lyophilized SkM-TnI stock by addition of 100 µl of de-ionized or distilled water. Mix gently until dissolved – **USE WITHIN 30 MINUTES OF RECONSTITUTION**. The concentration of SkM-TnI in the reconstituted stock is indicated on the vial label.
3. Label 7 polypropylene tubes as 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0 ng/ml.
4. Into the tube labeled 50 ng/ml, pipette the volume of **Standard Diluent** detailed on the SkM-TnI stock vial label. Then add the indicated volume of SkM-TnI stock (shown on the SkM-TnI stock vial label) and mix gently. This provides the 50 ng/ml standard.
5. Pipette 0.25 ml of **Standard Diluent** into the tubes labeled 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0 ng/ml
6. Prepare a 25 ng/ml standard by diluting and mixing 0.25 ml of the 50 ng/ml standard with 0.25 ml of standard diluent in the tube labeled 25 ng/ml. Similarly prepare the 12.5, 6.25, 3.125, 1.56 and 0.78 ng/ml standards by serial dilution.

NOTE: The reconstituted SkM-TnI standards should be used within 30 minutes of stock reconstitution. Discard the stock after use.

SAMPLE COLLECTION

Serum or plasma (EDTA) should be prepared as quickly as possible after blood collection and stored at 4°C. All samples should be similarly processed (i.e., storage times and temperatures should be the same for all samples). If samples cannot be assayed

within 1-2 hours of collection they should be frozen at -70°C and thawed only once prior to use.

SAMPLE PREPARATION

In studies at Life Diagnostics, Inc., we have encountered samples with very low (≤ 4 ng/ml) and high (> 500 ng/ml) levels of troponin-I. Depending on the level of troponin-I two different methods of sample preparation are recommended.

1. Low troponin-I levels: plasma or serum samples should be diluted with 1/3rd volume of **Sample Diluent** (i.e., 180 μl of serum or plasma should be diluted with 60 μl of sample diluent).
2. High troponin-I levels: If samples prepared as described in 1 above give absorbance values that exceed those of the 100 ng/ml standard, samples pre-diluted with Sample diluent as described above should be further diluted with **Standard diluent** (i.e., one volume of sample pre-diluted as described in 1 above, should be mixed directly with one or more volumes of Standard diluent).

We recommend that samples be assayed in duplicate. Wherever possible, all samples should be similarly diluted in order to avoid minor matrix differences.

PROCEDURAL NOTES

1. Standards and diluted samples should be prepared immediately prior to use and used within 30 minutes.
2. Pipetting of all standards, samples and conjugate into the microtiter plate should be completed within 10 minutes.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μl of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature ($18-25^{\circ}\text{C}$) for 45 minutes.
4. Remove the incubation mixture using either a plate washer or by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 5 times with 1x wash solution using a plate washer (400 μl /well). The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Add 100 μl of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature ($18-25^{\circ}\text{C}$) for 45 minutes.
9. Wash as detailed in 4 to 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
11. Dispense 100 μl of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature ($18-25^{\circ}\text{C}$) for 20 minutes.
13. Stop the reaction by adding 100 μl of Stop Solution to each well.
14. Gently mix. *It is important to make sure that all the blue color changes to yellow.*

15. Read the optical density at 450 nm with a microtiter plate reader *within 5 minutes*.
16. If absorbance values of samples exceed that of the 50 ng/ml standard, samples should be appropriately diluted and re-tested.

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for the standards and samples.
2. Construct a standard curve by plotting the A_{450} values obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the A_{450} values for each sample, determine the corresponding concentration of SkM-TnI (ng/ml) from the standard curve. If using graphing software, we suggest using a linear regression fit of the data.
5. Multiply the derived SkM-TnI concentrations by the dilution factor (i.e., 1.33, if the "low troponin-I level" dilution procedure was used) to obtain the actual SkM-TnI concentration.

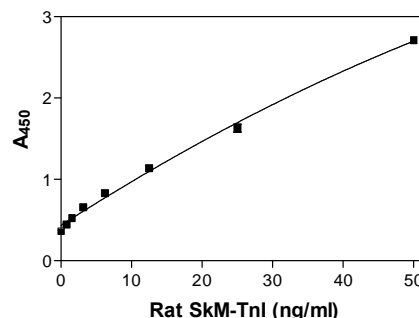
EXAMPLE OF STANDARD CURVE

Results of a typical standard curve with A_{450} plotted on the Y axis against cTnI concentrations on the X axis are shown below.

NOTE: This standard curve is for the purpose of illustration only.

SkM-TnI (ng/ml)	Absorbance (450 nm)
50	2.713
25	1.629
12.5	1.142
6.25	0.830
3.125	0.658
1.56	0.527
0.78	0.448
0	0.363

Typical Standard Curve



LIMITATIONS OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

RAT MYOGLOBIN ELISA

Life Diagnostics, Inc., Catalog Number: 2110-2-N

ELISA for the Determination of Myoglobin in Rat Serum, Plasma & Urine¹

STORAGE

Store standard at -20°C
STORE REMAINDER OF KIT AT 2 - 8°C

INTRODUCTION

Myoglobin is a heme protein found in both cardiac and skeletal muscle. Following myocardial necrosis associated with myocardial infarction (MI), myoglobin is one of the first markers to rise above normal levels. Studies with human subjects have shown that myoglobin increases measurably above baseline within 2-4 hours post-infarct, peaking at 9-12 hours, and returning to baseline within 24-36 hours. In the absence of skeletal muscle trauma or other factors associated with a noncardiac related increase in circulating myoglobin, myoglobin may be used as a marker for MI. Similarly, in the absence of cardiac damage, myoglobin may be used as a marker of skeletal muscle injury.

PRINCIPLE OF THE TEST

The Myoglobin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. A monoclonal anti-myoglobin antibody is used for solid phase immobilization (on the microtiter wells) and a polyclonal anti-myoglobin antibody conjugated to horseradish peroxidase is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the myoglobin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After one hour incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A TMB (Tetramethyl-benzidine) Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of myoglobin is proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- Anti-Myoglobin-coated microtiter wells, 96 wells
- Rat Myoglobin Stock (50 µl of 50 µg/ml) **Store at -20°C**
- Diluent, 12 ml
- Enzyme Conjugate Reagent, 11 ml
- 20x Wash Solution, 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

¹ A matrix effect may be observed with urine samples that results in slight differences in absorbance values relative to myoglobin diluted in the ELISA diluent. It is therefore recommended that wherever possible all urine samples within a particular study be similarly diluted prior to testing, thereby ensuring an accurate determination of relative myoglobin levels within the study.

Materials required but not provided:

- Precision pipettes
- Disposable pipette tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Graph paper (PC graphing software is optional but recommended)
- Plate shaker
- Microtiter plate reader

INSTRUMENTATION

A microtiter plate reader with an optical density range of 0-4 OD at 450 nm wavelength is required.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Label 8 polypropylene tubes as 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0 ng/ml.
2. Pipette 998 µl of diluent into the tube labeled 100 ng/ml
3. Pipette 100 µl of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.125, 1.56 and 0 ng/ml.
4. Briefly centrifuge or flick the myoglobin stock tube to ensure that the liquid contents are at the bottom of the tube.
5. Dilute 2 µl of the 50 µg/ml myoglobin stock into the 998 µl of diluent in the tube labeled 100 ng/ml. This provides a 100 ng/ml solution of myoglobin.
6. Prepare a 50 ng/ml stock by diluting and mixing 100 µl of the 100 ng/ml stock with 100 µl of diluent in the tube labeled 50 ng/ml. Similarly prepare the 25, 12.5, 6.25, 3.125, 1.56 ng/ml stocks by serial dilution.

SAMPLE COLLECTION

Serum, plasma and urine should be collected using standard techniques. Remove serum or plasma from the coagulated or packed cells within 60 minutes after collection. Plasma samples may be collected into tubes containing EDTA. Samples that cannot be assayed within 3 hours of collection should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed prior to testing.

SAMPLE PREPARATION

Samples may be tested undiluted or after dilution with diluent. The dilution factor should be determined empirically. On occasion a matrix effect may be observed with urine samples that may slightly increase or decrease absorbance values and we therefore strongly recommend that all urine samples within a particular study be similarly diluted. *Only 20 µl of*

sample is required per assay (2 x 20 µl, if samples are to be tested in duplicate).

PROCEDURAL NOTES

1. Standards should be prepared immediately prior to use and should be used within 30 minutes of preparation.
2. Pipetting of conjugate, standards and samples into the microtiter plate should be completed within 10 minutes.
3. We recommend that standards and samples be run in duplicate.
4. It is recommended that the wells be read within 5 minutes following addition of Stop Solution.

ASSAY PROCEDURE

1. Ensure that all reagents are at room temperature.
2. Secure the desired number of coated wells in the holder.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Dispense 20 µl of myoglobin standards and samples (in duplicate) into the appropriate wells.
5. Incubate at room temperature (18-25°C) on a plate shaker for one hour. Mix Gently (~100-150 rpm)
6. Remove the incubation mixture either with a plate washer or by flicking plate contents into a waste container.
7. Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 µl/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
9. Dispense 100 µl of TMB Reagent solution into each well. Gently mix for 5 seconds.
10. Incubate on a plate shaker at room temperature for 20 minutes. Mix Gently
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read absorbance at 450 nm with a microtiter well reader within 5 minutes.

CALCULATION OF RESULTS

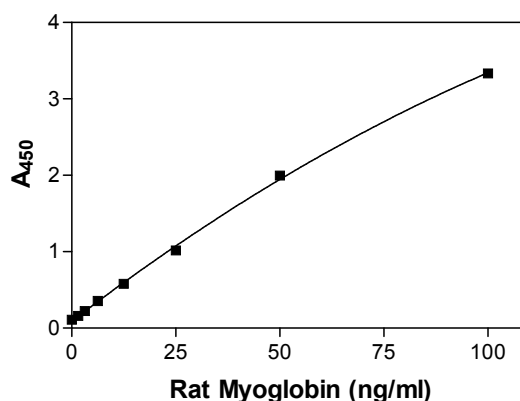
1. Calculate the mean absorbance value for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each sample to determine the corresponding concentration of myoglobin in ng/ml from the standard curve.
4. Multiply the derived value by the appropriate dilution factor if the test samples were diluted.
5. Graphing software, if available, should be used.

TYPICAL STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against myoglobin concentrations shown in the X axis are illustrated below. This standard curve is for illustrative purposes only, and should not be used to calculate unknowns. A standard curve should be run for each assay.

Myoglobin (ng/ml)	Absorbance (450 nm)
100	3.333
50	1.997
25	1.015
12.5	0.577
6.25	0.356
3.125	0.223
1.563	0.158

Typical Rat Myoglobin Standard Curve



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

MONKEY MYOGLOBIN ELISA

Life Diagnostics, Inc., Catalog Number: 2110-6-N

ELISA for the Determination of Myoglobin in Monkey Serum, Plasma & Urine¹

STORAGE

Store standard at -20°C
STORE REMAINDER OF KIT AT 2 - 8°C

INTRODUCTION

Myoglobin is a heme protein found in both cardiac and skeletal muscle. Following myocardial necrosis associated with myocardial infarction (MI), myoglobin is one of the first markers to rise above normal levels. Studies with human subjects have shown that myoglobin increases measurably above baseline within 2-4 hours post-infarct, peaking at 9-12 hours, and returning to baseline within 24-36 hours. In the absence of skeletal muscle trauma or other factors associated with a noncardiac related increase in circulating myoglobin, myoglobin may be used as a marker for MI. Similarly, in the absence of cardiac damage, myoglobin may be used as a marker of skeletal muscle injury.

PRINCIPLE OF THE TEST

The Myoglobin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. A monoclonal anti-myoglobin antibody is used for solid phase immobilization (on the microtiter wells) and a polyclonal anti-myoglobin antibody conjugated to horseradish peroxidase is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the myoglobin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After one hour incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A TMB (Tetramethyl-benzidine) Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of myoglobin is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- Anti-Myoglobin-coated microtiter wells, 96 wells
- Monkey Myoglobin Standard (100 µl, 50 µg/ml)²

¹ A matrix effect may be observed with urine samples that results in slight differences in absorbance values relative to myoglobin diluted in the ELISA diluent. It is therefore recommended that wherever possible all urine samples within a particular study be similarly diluted prior to testing, thereby ensuring an accurate determination of relative myoglobin levels within the study.

² Due to international/ import/export restrictions of monkey derived products, the myoglobin standard provided with this kit is of non monkey

- Diluent, 12 ml
- Enzyme Conjugate Reagent, 11 ml
- 20x Wash Solution, 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes
- Disposable pipette tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Graph paper (PC graphing software is optional but recommended)
- Plate shaker
- Microtiter plate reader

INSTRUMENTATION

A microtiter plate reader with an optical density range of 0-4 OD at 450 nm wavelength is required.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Label 8 polypropylene tubes as 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95 and 0 ng/ml.
2. Pipette 997.5 µl of diluent into the tube labeled 125 ng/ml
3. Pipette 100 µl of diluent into the tubes labeled 62.5, 31.25, 15.63, 7.81, 3.91, 1.95 and 0 ng/ml.
4. Briefly centrifuge or flick the myoglobin stock tube to ensure that the liquid contents are at the bottom of the tube.
5. Dilute 2.5 µl of the 50 µg/ml myoglobin stock into the 997.5 µl of diluent in the tube labeled 125 ng/ml. This provides a 125 ng/ml solution of myoglobin.
6. Prepare a 62.5 ng/ml stock by diluting and mixing 100 µl of the 125 ng/ml stock with 100 µl of diluent in the tube labeled 62.5 ng/ml. Similarly prepare the 31.25, 15.63, 7.81, 3.91 and 1.95 ng/ml stocks by serial dilution.

SAMPLE COLLECTION

Serum, plasma and urine should be collected using standard techniques. Remove serum or plasma from the coagulated or packed cells within 60 minutes after collection. Plasma

origin. The standard curve obtained with this material is identical to that obtained with monkey myoglobin.

samples may be collected into tubes containing EDTA. Samples that cannot be assayed within 3 hours of collection should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed prior to testing.

SAMPLE PREPARATION

Samples may be tested undiluted or after dilution with diluent. The dilution factor should be determined empirically. *Only 20 µl of sample is required per assay (2 x 20 µl, if samples are to be tested in duplicate).*

ASSAY PROCEDURE

1. Ensure that all reagents are at room temperature.
2. Secure the desired number of coated wells in the holder.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Dispense 20 µl of myoglobin standards and samples (in duplicate) into the appropriate wells.
5. Incubate at room temperature (18-25°C) on a plate shaker for one hour. Mix Gently (~100-150 rpm)
6. Remove the incubation mixture either with a plate washer or by flicking plate contents into a waste container.
7. Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 µl/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water drops.
9. Dispense 100 µl of TMB Reagent solution into each well. Gently mix for 5 seconds.
10. Incubate on a plate shaker at room temperature for 20 minutes. Mix Gently
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read absorbance at 450 nm with a microtiter well reader within 5 minutes.

CALCULATION OF RESULTS

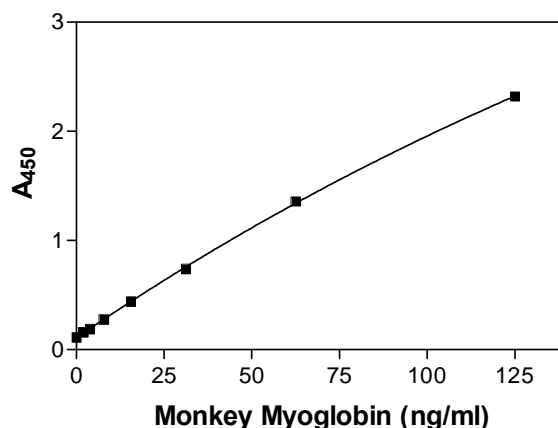
1. Calculate the mean absorbance value for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each sample to determine the corresponding concentration of myoglobin in ng/ml from the standard curve.
4. Multiply the derived value by the appropriate dilution factor if the test samples were diluted.
5. Graphing software, if available, should be used.

TYPICAL STANDARD CURVE

Results of a typical standard curve with optical density readings at 450 nm shown in the Y axis against myoglobin concentrations shown in the X axis are illustrated below. This standard curve is for illustrative purposes only, and should not be used to calculate unknowns. A standard curve should be run for each assay.

Myoglobin (ng/ml)	Absorbance (450 nm)
125	2.320
62.5	1.358
31.25	0.738
15.63	0.441
7.81	0.276
3.91	0.190
1.98	0.158
0	0.111

Typical Monkey Myoglobin Standard Curve



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

PIG MYOGLOBIN ELISA

Life Diagnostics, Inc., Catalog Number: 2110-5-N

ELISA for the Determination of Myoglobin in Pig Serum, Plasma & Urine¹

STORAGE

Store standard at -20°C
STORE REMAINDER OF KIT AT 2 - 8°C

INTRODUCTION

Myoglobin is a heme protein found in both cardiac and skeletal muscle. Following myocardial necrosis associated with myocardial infarction (MI), myoglobin is one of the first markers to rise above normal levels. Studies with human subjects have shown that myoglobin increases measurably above baseline within 2-4 hours post-infarct, peaking at 9-12 hours, and returning to baseline within 24-36 hours. In the absence of skeletal muscle trauma or other factors associated with a noncardiac related increase in circulating myoglobin, myoglobin may be used as a marker for MI. Similarly, in the absence of cardiac damage, myoglobin may be used as a marker of skeletal muscle injury.

PRINCIPLE OF THE TEST

The Myoglobin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. A monoclonal anti-myoglobin antibody is used for solid phase immobilization (on the microtiter wells) and a polyclonal anti-myoglobin antibody conjugated to horseradish peroxidase is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the myoglobin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After one hour incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A TMB (Tetramethyl-benzidine) Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of myoglobin is proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- Anti-Myoglobin-coated microtiter wells, 96 wells
- Pig Myoglobin Stock (50 µl of 15.625 µg/ml) **Store at -20°C**
- Diluent, 12 ml
- Enzyme Conjugate Reagent, 11 ml
- 20x Wash Solution, 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

¹ A matrix effect may be observed with urine samples that results in slight differences in absorbance values relative to myoglobin diluted in the ELISA diluent. It is therefore recommended that wherever possible all urine samples within a particular study be similarly diluted prior to testing, thereby ensuring an accurate determination of relative myoglobin levels within the study.

Materials required but not provided:

- Precision pipettes
- Disposable pipette tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Graph paper (PC graphing software is optional but recommended)
- Plate shaker
- Microtiter plate reader

INSTRUMENTATION

A microtiter plate reader with an optical density range of 0-4 OD at 450 nm wavelength is required.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Label 8 polypropylene tubes as 31.25, 15.63, 7.81, 3.91, 1.95, 0.98, 0.49 and 0 ng/ml.
2. Pipette 998 µl of diluent into the tube labeled 31.25 ng/ml
3. Pipette 100 µl of diluent into the tubes labeled 15.63, 7.81, 3.91, 1.95, 0.98, 0.49 and 0 ng/ml.
4. Briefly centrifuge or flick the myoglobin stock tube to ensure that the liquid contents are at the bottom of the tube.
5. Dilute 2 µl of the 15.625 µg/ml myoglobin stock into the 998 µl of diluent in the tube labeled 100 ng/ml. This provides a 31.25 ng/ml solution of myoglobin.
6. Prepare a 15.63 ng/ml stock by diluting and mixing 100 µl of the 31.25 ng/ml stock with 100 µl of diluent in the tube labeled 15.63 ng/ml. Similarly prepare the 7.81, 3.91, 1.95, 0.98, and 0.49 ng/ml stocks by serial dilution.

SAMPLE COLLECTION

Serum, plasma and urine should be collected using standard techniques. Remove serum or plasma from the coagulated or packed cells within 60 minutes after collection. Plasma samples may be collected into tubes containing EDTA. Samples that cannot be assayed within 3 hours of collection should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed prior to testing.

SAMPLE PREPARATION

Samples may be tested undiluted or after dilution with diluent. The dilution factor should be determined empirically. On occasion a matrix effect may be observed with urine samples that may slightly increase or decrease absorbance values and we therefore strongly recommend that all urine samples within a particular study be similarly diluted. *Only 20 µl of*

sample is required per assay (2 x 20 µl, if samples are to be tested in duplicate).

PROCEDURAL NOTES

1. Standards should be prepared immediately prior to use and should be used within 30 minutes of preparation.
2. Pipetting of conjugate, standards and samples into the microtiter plate should be completed within 10 minutes.
3. We recommend that standards and samples be run in duplicate.
4. It is recommended that the wells be read within 5 minutes following addition of Stop Solution.

ASSAY PROCEDURE

1. Ensure that all reagents are at room temperature.
2. Secure the desired number of coated wells in the holder.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Dispense 20 µl of myoglobin standards and samples (in duplicate) into the appropriate wells.
5. Incubate at room temperature (18-25°C) on a plate shaker for one hour. Mix Gently (~100-150 rpm)
6. Remove the incubation mixture either with a plate washer or by flicking plate contents into a waste container.
7. Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 µl/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
9. Dispense 100 µl of TMB Reagent solution into each well. Gently mix for 5 seconds.
10. Incubate on a plate shaker at room temperature for 20 minutes. Mix Gently
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read absorbance at 450 nm with a microtiter well reader within 5 minutes.

CALCULATION OF RESULTS

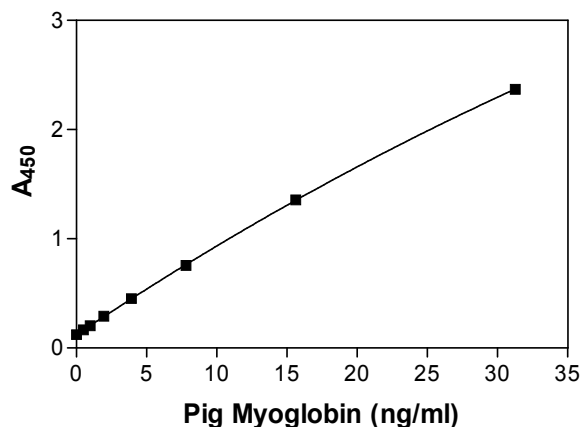
1. Calculate the mean absorbance value for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each sample to determine the corresponding concentration of myoglobin in ng/ml from the standard curve.
4. Multiply the derived value by the appropriate dilution factor if the test samples were diluted.
5. Graphing software, if available, should be used.

TYPICAL STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against myoglobin concentrations shown in the X axis are illustrated below. This standard curve is for illustrative purposes only, and should not be used to calculate unknowns. A standard curve should be run for each assay.

Myoglobin (ng/ml)	Absorbance (450 nm)
31.25	2.369
15.63	1.357
7.81	0.757
3.91	0.452
1.95	0.290
0.98	0.203
0.49	0.165
0	0.122

Typical Pig Myoglobin Standard Curve



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

DOG MYOGLOBIN ELISA

Life Diagnostics, Inc., Catalog Number: 2110-4-N

ELISA for the Determination of Myoglobin in Dog Serum, Plasma & Urine¹

STORAGE

Store standard at -20°C
STORE REMAINDER OF KIT AT 2 - 8°C

INTRODUCTION

Myoglobin is a heme protein found in both cardiac and skeletal muscle. Following myocardial necrosis associated with myocardial infarction (MI), myoglobin is one of the first markers to rise above normal levels. Studies with human subjects have shown that myoglobin increases measurably above baseline within 2-4 hours post-infarct, peaking at 9-12 hours, and returning to baseline within 24-36 hours. In the absence of skeletal muscle trauma or other factors associated with a noncardiac related increase in circulating myoglobin, myoglobin may be used as a marker for MI. Similarly, in the absence of cardiac damage, myoglobin may be used as a marker of skeletal muscle injury.

PRINCIPLE OF THE TEST

The Myoglobin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. A monoclonal anti-myoglobin antibody is used for solid phase immobilization (on the microtiter wells) and a polyclonal anti-myoglobin antibody conjugated to horseradish peroxidase is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the myoglobin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After one hour incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A TMB (Tetramethyl-benzidine) Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of myoglobin is proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- Anti-Myoglobin-coated microtiter wells, 96 wells
- Dog Myoglobin Stock (50 µl of 50 µg/ml) **Store at -20°C**
- Diluent, 12 ml
- Enzyme Conjugate Reagent, 11 ml
- 20x Wash Solution, 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

¹ A matrix effect may be observed with urine samples that results in slight differences in absorbance values relative to myoglobin diluted in the ELISA diluent. It is therefore recommended that wherever possible all urine samples within a particular study be similarly diluted prior to testing, thereby ensuring an accurate determination of relative myoglobin levels within the study.

Materials required but not provided:

- Precision pipettes
- Disposable pipette tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Graph paper (PC graphing software is optional but recommended)
- Plate shaker
- Microtiter plate reader

INSTRUMENTATION

A microtiter plate reader with an optical density range of 0-4 OD at 450 nm wavelength is required.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Label 8 polypropylene tubes as 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 0 ng/ml.
2. Pipette 398 µl of diluent into the tube labeled 250 ng/ml
3. Pipette 100 µl of diluent into the tubes labeled 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 0 ng/ml.
4. Briefly centrifuge or flick the myoglobin stock tube to ensure that the liquid contents are at the bottom of the tube.
5. Dilute 2 µl of the 50 µg/ml myoglobin stock into the 398 µl of diluent in the tube labeled 250 ng/ml. This provides a 250 ng/ml solution of myoglobin.
6. Prepare a 125 ng/ml stock by diluting and mixing 100 µl of the 250 ng/ml stock with 100 µl of diluent in the tube labeled 125 ng/ml. Similarly prepare the 62.5, 31.25, 15.63, 7.81 and 3.91 ng/ml stocks by serial dilution.

SAMPLE COLLECTION

Serum, plasma and urine should be collected using standard techniques. Remove serum or plasma from the coagulated or packed cells within 60 minutes after collection. Plasma samples may be collected into tubes containing EDTA. Samples that cannot be assayed within 3 hours of collection should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed prior to testing.

SAMPLE PREPARATION

Samples may be tested undiluted or after dilution with diluent. The dilution factor should be determined empirically. On occasion a matrix effect may be observed with urine samples that may slightly increase or decrease absorbance values and we therefore strongly recommend that all urine samples within a particular study be similarly diluted. *Only 20 µl of*

sample is required per assay (2 x 20 µl, if samples are to be tested in duplicate).

PROCEDURAL NOTES

1. Standards should be prepared immediately prior to use and should be used within 30 minutes of preparation.
2. Pipetting of conjugate, standards and samples into the microtiter plate should be completed within 10 minutes.
3. We recommend that standards and samples be run in duplicate.
4. It is recommended that the wells be read within 5 minutes following addition of Stop Solution.

ASSAY PROCEDURE

1. Ensure that all reagents are at room temperature.
2. Secure the desired number of coated wells in the holder.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Dispense 20 µl of myoglobin standards and samples (in duplicate) into the appropriate wells.
5. Incubate at room temperature (18-25°C) on a plate shaker for one hour. Mix Gently (~100-150 rpm)
6. Remove the incubation mixture either with a plate washer or by flicking plate contents into a waste container.
7. Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 µl/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
9. Dispense 100 µl of TMB Reagent solution into each well. Gently mix for 5 seconds.
10. Incubate on a plate shaker at room temperature for 20 minutes. Mix Gently
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read absorbance at 450 nm with a microtiter well reader within 5 minutes.

CALCULATION OF RESULTS

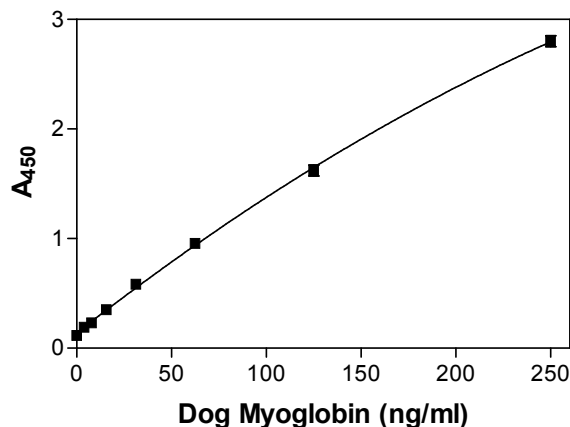
1. Calculate the mean absorbance value for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each sample to determine the corresponding concentration of myoglobin in ng/ml from the standard curve.
4. Multiply the derived value by the appropriate dilution factor if the test samples were diluted.
5. Graphing software, if available, should be used.

TYPICAL STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against myoglobin concentrations shown in the X axis are illustrated below. This standard curve is for illustrative purposes only, and should not be used to calculate unknowns. A standard curve should be run for each assay.

Myoglobin (ng/ml)	Absorbance (450 nm)
250	2.780
125	1.618
62.5	0.967
31.25	0.578
15.63	0.348
7.81	0.228
3.91	0.203
0	0.117

Typical Dog Myoglobin Standard Curve



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

RABBIT MYOGLOBIN ELISA

Life Diagnostics, Inc., Catalog Number: 2110-3-N

ELISA for the Determination of Myoglobin in Rabbit Serum, Plasma & Urine¹

STORAGE

Store standard at -20°C
STORE REMAINDER OF KIT AT 2 - 8°C

INTRODUCTION

Myoglobin is a heme protein found in both cardiac and skeletal muscle. Following myocardial necrosis associated with myocardial infarction (MI), myoglobin is one of the first markers to rise above normal levels. Studies with human subjects have shown that myoglobin increases measurably above baseline within 2-4 hours post-infarct, peaking at 9-12 hours, and returning to baseline within 24-36 hours. In the absence of skeletal muscle trauma or other factors associated with a noncardiac related increase in circulating myoglobin, myoglobin may be used as a marker for MI. Similarly, in the absence of cardiac damage, myoglobin may be used as a marker of skeletal muscle injury.

PRINCIPLE OF THE TEST

The Myoglobin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. A monoclonal anti-myoglobin antibody is used for solid phase immobilization (on the microtiter wells) and a polyclonal anti-myoglobin antibody conjugated to horseradish peroxidase is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the myoglobin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After one hour incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A TMB (Tetramethyl-benzidine) Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of myoglobin is proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- Anti-Myoglobin-coated microtiter wells, 96 wells
- Rabbit Myoglobin Stock (50 µl of 50 µg/ml) **Store at -20°C**
- Diluent, 12 ml
- Enzyme Conjugate Reagent, 11 ml
- 20x Wash Solution, 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

¹ A matrix effect may be observed with urine samples that results in slight differences in absorbance values relative to myoglobin diluted in the ELISA diluent. It is therefore recommended that wherever possible all urine samples within a particular study be similarly diluted prior to testing, thereby ensuring an accurate determination of relative myoglobin levels within the study.

Materials required but not provided:

- Precision pipettes
- Disposable pipette tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Graph paper (PC graphing software is optional but recommended)
- Plate shaker
- Microtiter plate reader

INSTRUMENTATION

A microtiter plate reader with an optical density range of 0-4 OD at 450 nm wavelength is required.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Label 8 polypropylene tubes as 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95 and 0 ng/ml.
2. Pipette 997.5 µl of diluent into the tube labeled 125 ng/ml
3. Pipette 100 µl of diluent into the tubes labeled 62.5, 31.25, 15.63, 7.81, 3.91, 1.95 and 0 ng/ml.
4. Briefly centrifuge or flick the myoglobin stock tube to ensure that the liquid contents are at the bottom of the tube.
5. Dilute 2.5 µl of the 50 µg/ml myoglobin stock into the 997.5 µl of diluent in the tube labeled 125 ng/ml. This provides a 125 ng/ml solution of myoglobin.
6. Prepare a 62.5 ng/ml stock by diluting and mixing 100 µl of the 125 ng/ml stock with 100 µl of diluent in the tube labeled 62.5 ng/ml. Similarly prepare the 31.25, 15.63, 7.81, 3.91 and 1.95 ng/ml stocks by serial dilution.

SAMPLE COLLECTION

Serum, plasma and urine should be collected using standard techniques. Remove serum or plasma from the coagulated or packed cells within 60 minutes after collection. Plasma samples may be collected into tubes containing EDTA. Samples that cannot be assayed within 3 hours of collection should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed prior to testing.

SAMPLE PREPARATION

Samples may be tested undiluted or after dilution with diluent. The dilution factor should be determined empirically. On occasion a matrix effect may be observed with urine samples that may slightly increase or decrease absorbance values and we therefore strongly recommend that all urine samples within a particular study be similarly diluted. *Only 20 µl of*

sample is required per assay (2 x 20 µl, if samples are to be tested in duplicate).

PROCEDURAL NOTES

- Standards should be prepared immediately prior to use and should be used within 30 minutes of preparation.
- Pipetting of conjugate, standards and samples into the microtiter plate should be completed within 10 minutes.
- We recommend that standards and samples be run in duplicate.
- It is recommended that the wells be read within 5 minutes following addition of Stop Solution.

ASSAY PROCEDURE

- Ensure that all reagents are at room temperature.
- Secure the desired number of coated wells in the holder.
- Dispense 100 µl of Enzyme Conjugate Reagent into each well.
- Dispense 20 µl of myoglobin standards and samples (in duplicate) into the appropriate wells.
- Incubate at room temperature (18-25°C) on a plate shaker for one hour. Mix Gently (~100-150 rpm)
- Remove the incubation mixture either with a plate washer or by flicking plate contents into a waste container.
- Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 µl/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
- Dispense 100 µl of TMB Reagent solution into each well. Gently mix for 5 seconds.
- Incubate on a plate shaker at room temperature for 20 minutes. Mix Gently
- Stop the reaction by adding 100 µl of Stop Solution to each well.
- Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
- Read absorbance at 450 nm with a microtiter well reader within 5 minutes.

CALCULATION OF RESULTS

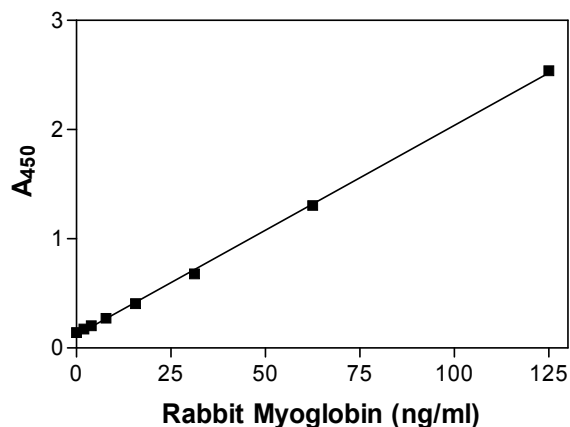
- Calculate the mean absorbance value for each set of reference standards and samples.
- Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
- Use the mean absorbance values for each sample to determine the corresponding concentration of myoglobin in ng/ml from the standard curve.
- Multiply the derived value by the appropriate dilution factor if the test samples were diluted.
- Graphing software, if available, should be used.

TYPICAL STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against myoglobin concentrations shown in the X axis are illustrated below. This standard curve is for illustrative purposes only, and should not be used to calculate unknowns. A standard curve should be run for each assay.

Myoglobin (ng/ml)	Absorbance (450 nm)
125	3.17
62.5	1.66
31.25	0.87
15.63	0.51
7.81	0.34
3.91	0.25
1.95	0.21
0	0.19

Typical Rabbit Myoglobin Standard Curve



LIMITATIONS OF THE PROCEDURE

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

MOUSE H-FABP ELISA KIT (HIGH SENSITIVITY)

Life Diagnostics, Inc., Catalog Number: 2310-1-HS

ELISA for the Quantitative Determination of Mouse Cardiac Fatty Acid-Binding Protein (H-FABP) in Serum and Plasma

INTRODUCTION

Fatty acid-binding proteins are cytoplasmic proteins of about 15 kDa that bind long chain fatty acids and play an important role in fatty acid metabolism. Different types of FABP have been detected including Heart FABP (H-FABP), Liver FABP and Intestinal FABP. Human cardiac muscle has a high content of H-FABP (10-20 mol % of cytoplasmic proteins) and H-FABP is a sensitive biomarker of myocardial necrosis that can be used to confirm or exclude a diagnosis of acute myocardial infarction (AMI). In AMI, H-FABP is rapidly released from damaged cardiomyocytes into the circulation due to its solubility and small size. Human clinical studies indicate that H-FABP levels are significantly elevated above threshold within 3 hours of AMI and subsequently return to normal values in 12 to 24 hours. H-FABP has also been identified as a potential serum biomarker for stroke that is superior to either neuron specific enolase or S100B. Our high sensitivity mouse H-FABP kit is offered as a tool for investigation of heart damage in mouse models of cardiovascular disease.

PRINCIPLE OF THE TEST

The H-FABP Quantitative test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses an affinity purified anti-mouse H-FABP antibody for solid phase (microtiter wells) immobilization and a horseradish peroxidase (HRP) conjugated anti-mouse H-FABP antibody for detection. The test sample is diluted and incubated with conjugate in the microtiter wells for 60 minutes. This results in mouse H-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes at room temperature, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of H-FABP is proportional to the optical density.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-mouse H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8).
- HRP Conjugate Reagent, 11 ml.
- Reference standard stock (lyophilized)
- 10x Diluent (25 ml)
- Wash Buffer (20x stock, 50 ml)
- TMB Reagent (One-Step) 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes and tips.
- Distilled water.
- Polypropylene microcentrifuge tubes (1.5 ml).
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Plate shaker with an approximate mixing speed of 100 rpm.
- Microtiter plate reader (450 nm wavelength) with an optical density range of 0-4 OD.
- Graph paper (PC graphing software is optional).

STORAGE OF TEST KIT AND INSTRUMENTATION

The reference standard stock provided with the kit should be frozen at or below -20°C on receipt. **The remainder of the kit should be stored at 2-8°C** and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date shown on the kit package, provided that the components are stored as described above.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. It may be necessary to dilute serum samples with the assay diluent in order to obtain values within the standard range.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

WASH BUFFER PREPARATION

The wash buffer is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The mouse H-FABP standard is provided as a lyophilized stock. Reconstitute with the volume of diluent indicated on the vial label. Mix gently until the contents of the vial dissolve. **The reconstituted standard should be aliquoted and frozen at or below -20°C after reconstitution if additional use is intended.**
2. Prepare a working 20 ng/ml standard according to the instructions on the reference standard vial label.
3. Label 7 polypropylene or glass tubes as 10, 5, 2.5, 1.25, 0.625, 0.313 and 0 ng/ml and pipette 250 µl of diluent into each tube.
4. Into the tube labeled 10 ng/ml, pipette and mix 250 µl of the 20 ng/ml standard. This provides the 10 ng/ml standard.

- Similarly prepare the 5, 2.5, 1.25, 0.625 and 0.313 ng/ml standards by serial dilution.

SAMPLE PREPARATION

Serum or EDTA plasma may be used in the assay. Avoid use of heparin plasma. Baseline levels of H-FABP are in the range of 1 – 2 ng/ml and can increase to 30 ng/ml or higher in rats following cardiac injury¹. Similar findings are expected for mice. We recommend that samples be diluted 5-fold prior to assay. This may be achieved by mixing 50 μ l of each test sample with 200 μ l of 1x diluent.

ASSAY PROCEDURE

- Secure the desired number of coated wells in the holder.
- Dispense 100 μ l of standards and samples into the wells (we recommend that samples be tested in duplicate).
- Add 100 μ l of enzyme conjugate reagent into each well.
- Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 60 minutes.
- Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 μ l/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
- Dispense 100 μ l of TMB Reagent into each well.
- Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
- Stop the reaction by adding 100 μ l of Stop Solution to each well.
- Gently mix. *It is important to make sure that all the blue color changes to yellow.*
- Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

CALCULATION OF RESULTS

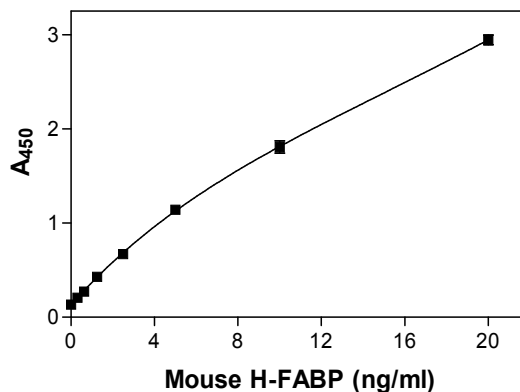
- Calculate the average absorbance values (A_{450}) for each set of reference standards, and samples.
- Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
- Using the mean absorbance value for each sample, determine the corresponding concentration of H-FABP in ng/ml from the standard curve.
- Multiply the derived concentrations by the dilution factor to determine the actual concentration of H-FABP in the sample.
- PC graphing software may be used for the above steps.
- If the OD_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown on the Y axis against H-FABP concentrations shown on the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

H-FABP (ng/ml)	Absorbance (450 nm)
20	2.946
10	1.808
5	1.142
2.5	0.670
1.25	0.430
0.625	0.272
0.313	0.206
0	0.135

Representative Mouse H-FABP Standard Curve



LIMITATIONS OF THE PROCEDURE

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

REFERENCES

- Yoshitani K, Qing M, Zhu B, Burkhard Mackensen G and Podgoreanu MV. Strain-Specific Differences in Sensitivity to Myocardial Injury after Cardioplegic Arrest in the Rat. Am. Soc. Anesthesiol., Annual Meeting Abstracts, Oct. 15 2007, A1326

RAT CK-MM ELISA KIT

Life Diagnostics, Inc., Catalog Number: 2112-2

ELISA for the Determination of Rat Creatine Kinase MM (CK-MM) in Serum or Plasma

Please Read All Instructions Before Starting The ELISA

INTRODUCTION

Creatine kinase (CK) is a key metabolic enzyme. It is a dimer of two subunits, each with molecular weights of approximately 43 kDa. Two different subunits occur, M and B. The CK holoenzyme therefore exists as MM and BB homodimers and an MB heterodimer. The MM and BB isoforms are expressed primarily in skeletal muscle and brain respectively while both MB and MM are expressed in heart. The relatively high expression levels of the MB isoform in heart explain its use as an established biomarker for heart disease. Likewise, CK-MM can be used as a specific biomarker for skeletal muscle injury.

PRINCIPLE OF THE TEST

The rat CK-MM ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified CK-MM antibodies for solid phase immobilization and a horseradish peroxidase (HRP) conjugated CK-MM monoclonal antibody for detection. Standards and diluted test samples are incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. As a result, CK-MM molecules are sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of CK-MM is proportional to the optical density of the test sample and actual concentrations are determined by reference to a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-rat CK-MM antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- 2x HRP Conjugate, 6 ml
- Reference rat CK-MM stock (lyophilized)
- Wash Buffer (20x stock, 50 ml)
- Diluent (50 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes

- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- Plate reader with an optical density range of 0-4 at 450nm
- Graph paper (PC graphing software is optional).

STORAGE OF TEST KIT

The kit should be stored at 2-8°C and the microtiter strips should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase provided that the components are stored as described above.

GENERAL INSTRUCTIONS

All reagents should be allowed to reach room temperature (18-25°C) before use.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

HRP CONJUGATE PREPARATION

The HRP conjugate is provided as a 2x stock. Prior to use estimate the final volume of HRP conjugate required for your assay and dilute one (1) volume of the 2x stock with one (1) volume of diluent. Typically, we prepare 1 ml of conjugate for each 8-well strip used in the assay. This is prepared by mixing 0.5 ml of 2x HRP conjugate with 0.5 ml of diluent. Mix gently prior to use. Prepare the working conjugate no more than one hour in advance.

STANDARD PREPARATION

1. The reference CK-MM stock is provided in lyophilized form. Reconstitute with diluent as directed on the vial label and mix gently until dissolved. (**the reconstituted standard remains stable for 1 day at 2-8°C**).
2. Label 5 polypropylene or glass tubes as 50, 25, 12.5, 6.25 and 3.13 ng/ml.
3. Prepare the 50 ng/ml standard as described on the reference stock vial label.
4. Dispense 250 µl of diluent into the tubes labeled 25, 12.5, 6.25 and 3.13 ng/ml.
5. Pipette 250 µl of the 50 ng/ml CK-MM standard into the tube labeled 25 ng/ml and mix. This provides the working 25 ng/ml CK-MM standard.
6. Similarly prepare the 12.5, 6.25 and 3.13 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: CK-MM is usually below the detection level of the kit in samples from animals without skeletal muscle injury. However, after skeletal muscle injury levels can increase to

8000 ng/ml or greater. Undiluted serum or plasma must not be used in the ELISA. We recommend that samples be diluted at least 10-fold or more. This eliminates interference caused by serum/plasma components. Assuming that a dilution of 10-fold is used; samples should be treated as follows:

1. Dispense 225 μ l of diluent into separate tubes.
2. Pipette and mix 25 μ l of the serum/plasma sample into the tube containing 225 μ l of diluent. This provides a 10 fold diluted sample.
3. Repeat this procedure for each sample to be tested

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μ l of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
4. Remove the incubation mixture using either a plate washer or by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 5 times with 1x wash solution. This should preferentially be performed using a plate washer (400 μ l/well). If a plate washer is not available use a squirt bottle. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Add 100 μ l of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
9. Wash as detailed in 4 to 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
11. Dispense 100 μ l of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
13. Stop the reaction by adding 100 μ l of Stop Solution to each well.
14. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
15. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CK-MM in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of CK-MM in the serum/plasma sample.
5. PC graphing software may be used for the above steps. We recommend a point-to-point "fit" of the data because of the

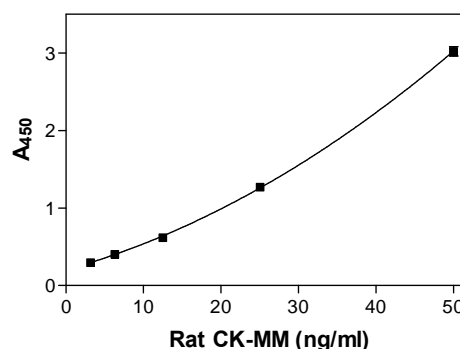
concave shape of the standard curve. Alternatively, fitting to a polynomial second order equation may work well.

6. If the A_{450} values of samples exceed that of the high standard the samples should be further diluted and re-tested. Samples with absorbance values below the lowest standard should be assigned a value of $\leq 3.13 \times A$ ng/ml, where A represents the dilution factor used for the sample.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against CK-MM concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

CK-MM (ng/ml)	Absorbance (450 nm)
50	3.021
25	1.269
12.5	0.618
6.25	0.406
3.13	0.299



LIMITATIONS OF THE PROCEDURE

1. Do not use samples at a dilution less than 10-fold (i.e., do not use a dilution of 5-fold). High serum content of the samples can interfere with the ELISA.
2. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

SPECIFICITY

Rat CK-BB and rat CK-MB were not available when this kit was developed and specificity was therefore investigated using purified human CK-MM, CK-MB and CK-BB. Human CK-MM was strongly recognized but CK-BB was not recognized at concentrations up to 10 micrograms/ml. The ELISA was ~400-fold more selective for CK-MM compared to CK-MB.

RAT & MOUSE SKELETAL MUSCLE MYOSIN LIGHT CHAIN-1 ELISA KIT

Life Diagnostics, Inc., Cat. No. 2325-2

ELISA for Determination of Rat and Mouse Skeletal Muscle Myosin Light Chain-1 (SMLC-1)

STORAGE CONDITIONS

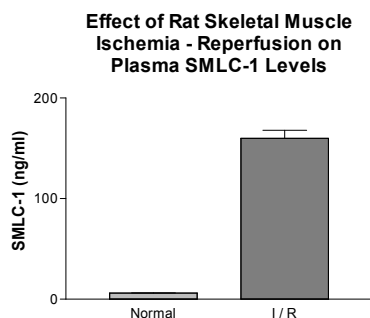
Store the kit at 2-8°C. Keep the microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

EXPIRATION

The kit expiration date (six months from the date of shipment) is indicated on the package label

BACKGROUND

Myosin light chains are released into the circulation following muscle injury and provide useful biomarkers of muscle damage. Myosin light chain-1 (MLC-1) is expressed as different but immunologically related isoforms in skeletal muscle and heart. The antibodies used in this ELISA kit also cross-react with cardiac MLC-1 (CMLC-1). However, as indicated in the figure below, the assay provides an excellent tool for assessment of skeletal muscle injury in the absence of cardiac damage¹.



PRINCIPLE OF THE ASSAY

The SMLC-1 ELISA uses two different MLC-1 monoclonal antibodies. One is used for solid phase immobilization (on the microtiter wells). The second is conjugated to horse radish peroxidase (HRP) and is used for detection. The sample is diluted with diluent as necessary and 100 µl aliquots of samples and standards are incubated in the microtiter wells for one hour. The wells are then washed and anti-SMLC-1 HRP conjugate is added and incubated in the wells for one hour. SMLC-1 molecules are thereby sandwiched between the solid phase and HRP-conjugated antibodies. After washing to remove unbound HRP conjugate a solution of tetramethylbenzidine (TMB), an HRP substrate, is then added to the wells and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped by addition of 1N HCl changing the color to yellow. The concentration

¹ Cardiac injury may be assessed using rat or mouse cardiac troponin-I ELISA kits available from Life Diagnostics, Inc.

of SMLC-1 is proportional to the absorbance at 450 nm and is derived from a standard curve.

REAGENTS AND MATERIALS PROVIDED

- Anti MLC-1-Coated Wells (1 plate, 96 wells, 12 x 8-well strips)
- SMLC-1 Stock: Lyophilized rat SMLC-1
- Diluent (25 ml)
- 20x Wash Solution (50 ml)
- SMLC-1 HRP Conjugate (11 ml)
- TMB Reagent (11 ml)
- Stop Solution (11 ml)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Pipettes: P-10, P-200 & P-1000 or equivalent
3. Disposable pipette tips
4. Microtiter well reader capable of reading OD at 450 nm
5. Vortex mixer
6. Absorbent paper
7. Graph paper or appropriate PC graphing software
8. Polypropylene microcentrifuge tubes (1.5 ml)

WARNINGS AND PRECAUTIONS

1. Avoid contact with Stop Solution (1N HCl). It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
2. Do not use reagents after expiration date and do not mix or use components from different kits.
3. Replace caps on reagents immediately. Do not switch caps.
4. Do not pipette reagents by mouth.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Equilibrate kit components to room temperature before use.
2. Reconstitute the lyophilized SMLC-1 stock as directed on the vial label. Mix gently several times over a period of 5-10 minutes
3. Label 6 polypropylene tubes as 50, 25, 12.5, 6.25, 3.125 and 1.56 ng/ml.
4. Into the tube labeled 50 ng/ml, pipette the volume of diluent detailed on the SMLC-1 stock vial label. Then add the indicated volume of SMLC-1 stock (shown on the vial label) and mix gently. This provides the 50 ng/ml standard.
5. Pipette 0.25 ml of standard diluent into the tubes labeled 25, 12.5, 6.25, 3.125 and 1.56 ng/ml.
6. Prepare a 25 ng/ml standard by diluting and mixing 0.25 ml of the 50 ng/ml standard with 0.25 ml of diluent in the tube

labeled 25 ng/ml. Similarly prepare the 12.5, 6.25, 3.125 and 1.56 ng/ml standards by serial dilution.

NOTE: The reconstituted SMLC-1 stock should be frozen immediately after use. It remains stable in frozen form for at least 6 months at -70°C. Discard the working 50 – 1.56 ng/ml standards after use.

SAMPLE COLLECTION AND PREPARATION

Plasma and serum should be prepared as quickly as possible after blood collection and stored at 4°C. All samples should be similarly processed (i.e., storage times and temperatures should be the same for all samples). If samples cannot be assayed within 4 hours of collection they should be frozen at -70°C and thawed only once prior to use.

We recommend that samples be assayed in duplicate. Optimum sample dilution should be determined by the end user. Samples should only be diluted with diluent supplied with the kit.

PROCEDURAL NOTES

- Standards and diluted plasma samples should be prepared immediately prior to use and used within 30 minutes.
- Pipetting of all standards, samples and conjugate into the microtiter plate should be completed within 5 minutes.
- It is recommended that the wells be read within 5 minutes following addition of Stop Solution.

ASSAY PROCEDURE

- Secure the desired number of coated wells in the holder.
- Dispense 100 µl of standards and diluted samples into appropriate wells.
- Thoroughly mix and incubate on an orbital shaker (150 rpm) at room temperature (18-25°C) for one hour.
- Remove the incubation mixture using a plate washer or by flicking the plate contents into a waste container.
- Wash and empty the microtiter wells 5 times with 1x wash solution. This should preferably be performed with a plate washer (400 µl/well). Alternatively a squirt bottle may be used. The entire wash procedure should be performed as quickly as possible.
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
- Dispense 100 µl of SMLC-1 HRP conjugate into each well.
- Incubate on an orbital shaker (150 rpm) at room temperature (18-25°C) for one hour.
- Wash the microtiter wells as directed in steps 4 -6 above.
- Dispense 100 µl of TMB Reagent into each well.
- Incubate at room temperature for 20 minutes on an orbital shaker at ~150 rpm.
- Stop the reaction by adding 100 µl of Stop Solution to each well.
- Gently mix. **It is important to make sure that all the blue color changes to yellow.**
- Read absorbance at 450 nm with a microtiter well reader within 5 minutes. **Please Note: Due to plate reader differences, the high standard absorbance values may occasionally be out**

of range. If this occurs, absorbance values may be determined at 405 nm instead.

- If the absorbance values of the samples exceed those of highest standard the samples should be further diluted with diluent and re-tested. For practical purposes, samples with absorbance values below that of the lowest standard should be assigned a zero value.

CALCULATION OF RESULTS

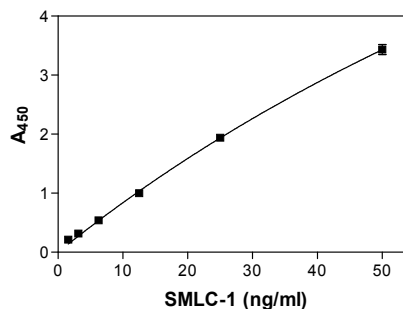
- Calculate the mean absorbance value (A_{450}) for the standards and samples.
- Construct a standard curve by plotting the A_{450} values obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
- Using the A_{450} values for each sample, determine the corresponding concentration of SMLC-1 (ng/ml) from the standard curve. If using graphing software, we suggest a point-to-point, or two site binding (hyperbola) fit of the data. The end user should choose the best data fit for the standard curve.
- Multiply the derived SMLC-1 concentrations by the dilution factor to obtain the actual SMLC-1 concentration.

EXAMPLE OF STANDARD CURVE

Results of a typical standard curve with A_{450} plotted on the Y axis against SMLC-1 concentrations on the X axis are shown below. **NOTE:** This standard curve is for the purpose of illustration only.

SMLC-1 (ng/ml)	Absorbance (450 nm)
50	3.433
25	1.937
12.5	1.000
6.25	0.538
3.13	0.317
1.56	0.211

Typical SMLC-1 Std Curve



LIMITATIONS OF THE PROCEDURE

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

MONKEY CARDIAC FATTY ACID BINDING PROTEIN ELISA KIT

Life Diagnostics, Inc., Catalog Number: 2310-7

ELISA for the Quantitative Determination of Cardiac Fatty Acid Binding Protein (H-FABP) in Monkey Serum

INTRODUCTION

Fatty acid-binding proteins (FABP) are cytosolic proteins of about 15 kDa. They bind long chain fatty acids and play an important role in fatty acid metabolism. Heart, liver, and intestinal FABP isoforms exist. Heart has a high content of FABP (10-20 mol % of cytoplasmic proteins), and heart FABP (H-FABP) has proved to be a sensitive biomarker of myocardial necrosis in humans. H-FABP is rapidly released into the circulation from damaged cardiac muscle. In humans serum levels increase significantly within 1-4 hours of muscle injury and return to normal within 12 to 24 hours. Because H-FABP is also expressed in skeletal muscle, it is necessary to exclude or control for skeletal muscle injury before ascribing H-FABP elevations to cardiac injury. However, in the absence of cardiac injury H-FABP is a useful biomarker of skeletal muscle injury. Validation studies at Life Diagnostics Inc., revealed basal monkey H-FABP levels of ~ 20 ng/ml with levels in excess of 400 ng/ml in animals with muscle injury.

PRINCIPLE OF THE TEST

The monkey H-FABP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses a mouse monoclonal anti-H-FABP antibody for solid phase (microtiter wells) immobilization and a different horseradish peroxidase (HRP) conjugated mouse monoclonal anti-H-FABP antibody for detection. Standards and diluted samples are incubated with the HRP conjugate in the microtiter wells for 60 minutes. This results in H-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies, and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of H-FABP is proportional to optical density. H-FABP concentrations are determined by reference to a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-H-FABP antibody coated 96 well plate (12 strips of 8 wells)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard¹ (lyophilized)
- Diluent, 50 ml
- 20x Wash Solution, 50 ml

¹Due to international import/export restrictions of monkey derived products the reference standard provided with this kit is of non monkey origin. The standard curve obtained with this material is identical to that obtained with native monkey H-FABP.

- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips.
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an OD range of 0-4 OD
- Graph paper (PC graphing software is optional)

STORAGE OF TEST KIT

The lyophilized reference standard must be stored at or below **-20°C on receipt**. The remainder of the kit should be stored at 2-8°C, and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase provided that the components are stored as described above.

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (18- 25°C) before use.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The reference standard is provided in lyophilized form. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved (**the reconstituted standard should be aliquoted and frozen at or below -20°C if further use is intended**).
2. Label 8 polypropylene or glass tubes as 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, and 0 ng/ml.
3. Into the tube labeled 250 ng/ml, pipette the volume of diluent detailed on the reference standard vial label. Then add the indicated volume of reconstituted standard and mix gently. This provides the 250 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 125, 62.5, 31.3, 15.6, 7.8, 3.9, and 0 ng/ml.
5. Pipette 250 µl of the 250 ng/ml H-FABP standard into the tube labeled 125 ng/ml H-FABP standard. This provides the working 125 ng/ml H-FABP standard. Similarly prepare the 62.5, 31.3, 15.6, 7.8, and 3.9 ng/ml standards by serial dilution.

SAMPLE PREPARATION

We suggest that samples initially be tested after a 5-fold dilution with the diluent provided with the kit.

1. Dispense 240 µl of 1x diluent into separate tubes.

- Pipette and mix 60 μl of each serum sample into a tube containing 240 μl of diluent. This provides a 5-fold diluted sample.

ASSAY PROCEDURE

- Secure the desired number of coated wells in the holder.
- Dispense 100 μl of standards and samples into the wells (we recommend that standards and samples be tested in duplicate).
- Add 100 μl of enzyme conjugate reagent into each well.
- Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 60 minutes.
- Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 μl /well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
- Dispense 100 μl of TMB Reagent into each well.
- Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
- Stop the reaction by adding 100 μl of Stop Solution to each well.
- Gently mix. It is important to make sure that all the blue color changes to yellow.
- Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

CALCULATION OF RESULTS

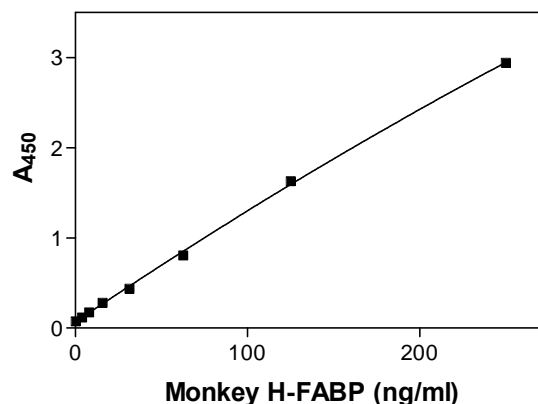
- Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
- Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
- Using the mean absorbance value for each sample, determine the corresponding concentration of H-FABP in ng/ml from the standard curve.
- Multiply the derived concentrations by the dilution factor to determine the actual concentration of H-FABP in the serum sample.
- If available, PC graphing software may be used for the above steps.
- If the A_{450} values of samples fall outside the standard curve samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450 nm on the Y axis against H-FABP concentration on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns.

H-FABP (ng/ml)	Absorbance (450 nm)
250.0	2.94
125.0	1.631
62.5	0.806
31.3	0.439
15.6	0.28
7.8	0.176
3.9	0.118
0.0	0.075

Typical Monkey H-FABP Standard Curve



LIMITATIONS OF THE PROCEDURE

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- All steps should be completed as quickly as accuracy allows.
- This kit is intended for use with serum, not plasma.

RABBIT CARDIAC FATTY ACID-BINDING PROTEIN (H-FABP) ELISA TEST KIT

Life Diagnostics, Inc., Catalog Number: 2310-6

Enzyme Immunoassay for the Quantitative Determination of Rabbit Cardiac Fatty Acid-Binding Protein (H-FABP) in Serum

Store Lyophilized Standard Stock at -20°C
Store the remainder of the kit at 2 to 8°C

INTRODUCTION

Fatty acid-binding proteins (FABP's) are a class of cytoplasmic proteins of about 15 kDa that bind and transport long chain fatty acids. Different isoforms of FABP have been identified including heart FABP (H-FABP), liver FABP and intestinal FABP. Cardiac muscle has high content of H-FABP (10-20 mol % of cytoplasmic proteins) and H-FABP is a sensitive biomarker of myocardial injury. Following cardiac damage, H-FABP is rapidly released from damaged cardiomyocytes into the circulation due to its solubility and small size. Studies in rabbits have demonstrated that H-FABP levels peak as early as one hour after cardiac damage. Our rabbit H-FABP kit is offered as a tool for investigation of heart damage in rabbit models of cardiovascular disease.

PRINCIPLE OF THE TEST

The H-FABP test is a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses an affinity purified anti-rabbit H-FABP antibody for solid phase (microtiter wells) immobilization and a horseradish peroxidase (HRP) conjugated anti-rabbit H-FABP antibody for detection. The test sample is diluted and incubated with conjugate in the microtiter wells for 60 minutes. This results in H-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes at room temperature, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of H-FABP is proportional to the optical density.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-rabbit H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8).
- HRP Conjugate Reagent, 11 ml.
- Reference standard stock (lyophilized), 2
- Diluent (25 ml)
- Wash Buffer (20x stock, 50 ml)
- TMB Reagent (One-Step) 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes and tips.
- Deionized water

- Polypropylene microcentrifuge tubes (1.5 ml)
- Vortex mixer or equivalent
- Absorbent paper or paper towel
- Plate shaker with an approximate mixing speed of 100 rpm
- Microtiter plate reader (450 nm wavelength) with an optical density range of 0-4 OD.
- Graph paper (PC graphing software is optional).

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by approved techniques (plasma may be used at the researcher's discretion but may require dilution with assay diluent in order to avoid matrix effects).

STORAGE OF TEST KIT AND INSTRUMENTATION

The reference standard stocks provided with the kit should be stored at or below -20°C on receipt. **The remainder of the kit should be stored at 2-8°C** and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date shown on the kit package, provided that the components are stored as described above.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. It may be necessary to dilute serum samples with the assay diluent in order to obtain values within the standard range.

WASH BUFFER PREPARATION

The wash buffer is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The rabbit H-FABP standard is provided as a lyophilized stock. Reconstitute with the volume of diluent indicated on the vial label. Mix gently until the contents of the vial dissolve. This provides the working 100 ng/ml standard. **The reconstituted standard should be aliquoted and frozen at or below -20°C after reconstitution if additional use is intended.**
2. Label 7 polypropylene or glass tubes as 50, 25, 12.5, 6.25, 3.125, 1.56 and 0 ng/ml and pipette 250 µl of diluent into each tube.
3. Into the tube labeled 50 ng/ml, pipette and mix 250 µl of the 100 ng/ml standard. This provides the 50 ng/ml standard.
4. Similarly prepare the 25, 12.5, 6.25, 3.125, and 1.56 ng/ml standards by serial dilution.

SAMPLE PREPARATION

Serum should be used in the assay. We found baseline levels of rabbit H-FABP to be approximately 2 ng/ml. Following ischemia reperfusion injury, levels increased to ~90 ng/ml one hour after reperfusion. It may be necessary to dilute samples with the assay diluent in order to obtain samples within range of the standard curve. If so, dilute samples with the diluent provided.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μ l of standards and samples into the wells (we recommend that samples be tested in duplicate).
3. Add 100 μ l of enzyme conjugate reagent into each well.
4. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 60 minutes.
5. Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 μ l/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Dispense 100 μ l of TMB Reagent into each well.
8. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
9. Stop the reaction by adding 100 μ l of Stop Solution to each well.
10. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
11. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes. In the event that the high standard exceeds 4 OD units, either eliminate the 100 ng/ml standard from the analysis or measure optical density at 405 nm. Absorbance values at 405 nm will be lower but the precision of the assay is not significantly diminished.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of reference standards, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of H-FABP in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of H-FABP in the sample.
5. PC graphing software may be used for the above steps.
- 6.

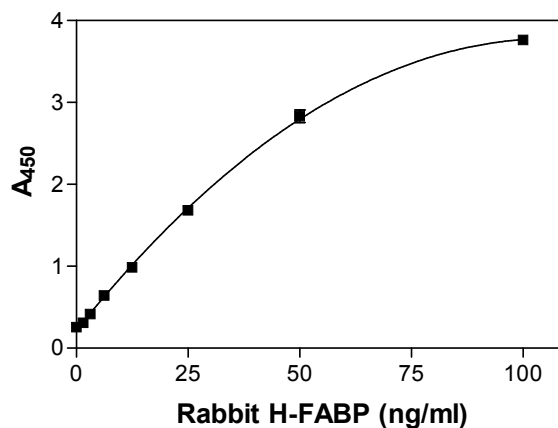
TYPICAL STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown on the Y axis against H-FABP concentrations shown on the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user

should obtain his or her data and standard curve in each experiment.

H-FABP (ng/ml)	Absorbance (450 nm)
100	3.762
50	2.831
25	1.681
12.5	0.986
6.25	0.641
3.125	0.415
1.56	0.307
0	0.255

Representative Rabbit H-FABP Standard Curve



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

PIG CARDIAC FATTY ACID BINDING PROTEIN ELISA KIT

Life Diagnostics, Inc., Catalog Number: 2310-5

Enzyme Immunoassay for the Quantitative Determination of Pig Cardiac Fatty Acid Binding Protein (H-FABP)

INTRODUCTION

Fatty acid-binding proteins (FABP) are cytosolic proteins of about 15 kD. They bind long chain fatty acids and play an important role in fatty acid metabolism. Heart, liver and intestinal FABP isoforms exist. Heart has a high content of FABP (10-20 mol % of cytoplasmic proteins) and heart FABP (H-FABP) has proved to be a sensitive biomarker of myocardial necrosis in humans. H-FABP is rapidly released into the circulation from damaged cardiac muscle. Serum/plasma levels are significantly increased within 1-4 hours of muscle injury and values return to normal within 12 to 24 hours. Because H-FABP is also expressed in skeletal muscle, it is necessary to exclude or control for skeletal muscle injury before ascribing H-FABP elevations to cardiac injury.

PRINCIPLE OF THE TEST

The pig H-FABP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-pig H-FABP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-pig H-FABP antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 30 minutes. This results in H-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. H-FABP concentration is proportional to the optical density of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-pig H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard (lyophilized)
- Diluent (25 ml)
- 20x Wash Solution (50 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips.
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer.
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm

- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an OD range of 0-4 OD
- Graph paper (PC graphing software is optional)

STORAGE OF TEST KIT

The unused kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

All reagents should be allowed to reach room temperature (18-25°C) before use.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The pig H-FABP standard is provided in lyophilized form. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved to obtain a 500 ng/ml stock (***the reconstituted 500 ng/ml standard is stable for 1 week at 2-8°C but should be aliquoted and frozen at -20°C after reconstitution if further use is intended.***)
2. Label 7 polypropylene or glass tubes as 25, 12.5, 6.25, 3.13, 1.56, 0.787 and 0 ng/ml
3. Dispense 570 µl of diluent into the tube labeled 25 ng/ml and 300 µl of diluent into the remaining tubes.
4. Pipette 30 µl of the 500 ng/ml H-FABP standard into the tube labeled 25 ng/ml and mix. This provides the working 25 ng/ml H-FABP standard.
5. Prepare a 12.5 ng/ml standard by diluting and mixing 300 µl of the 25 ng/ml standard with 300 µl of diluent in the tube labeled 12.5 ng/ml. Similarly prepare the 6.25, 3.13, 1.56, and 0.78 ng/ml standards by serial dilution.

SAMPLE PREPARATION

1. Pig serum or plasma samples may need to be diluted prior to assay in order to obtain values within range of the standard curve. The dilution factor must be determined empirically. We suggest that the researcher chooses a sample likely to have the highest H-FABP level and run an initial test with that sample to determine an optimum dilution factor. Other samples should subsequently be tested at that dilution.
2. Dilution should be performed with the yellow diluent provided with the kit.
3. If samples are to be tested in duplicate a final volume of 250 µl of diluted sample is sufficient.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.

4. Remove the incubation mixture either with a plate washer or by flicking plate contents into a waste container.
5. Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 μ l/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Add 100 μ l of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 30 minutes.
9. Wash as detailed in 4 and 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
11. Dispense 100 μ l of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
13. Stop the reaction by adding 100 μ l of Stop Solution to each well.
14. Gently mix. It is important to make sure that all the blue color changes to yellow.
15. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

CALCULATION OF RESULTS

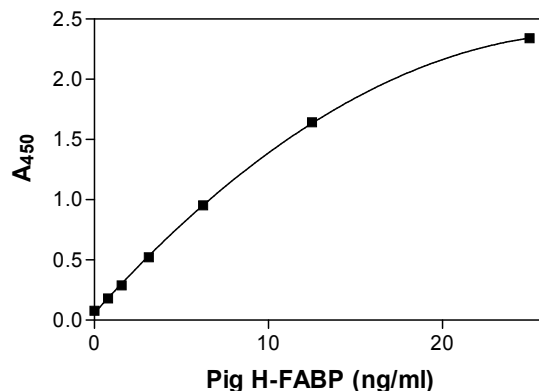
1. Calculate the average absorbance values (A_{450}) for each set of reference standards, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of H-FABP in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of H-FABP in the serum/plasma sample.
5. If available, PC graphing software may be used for the above steps.
6. If the OD_{450} values of samples fall outside the standard curve samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against H-FABP concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns.

H-FABP (ng/ml)	Absorbance (450 nm)
25	2.341
12.5	1.643
6.25	0.954
3.13	0.523
1.56	0.289
0.78	0.180
0	0.079

Typical Pig H-FABP Standard Curve



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

DOG CARDIAC FATTY ACID BINDING PROTEIN ELISA KIT

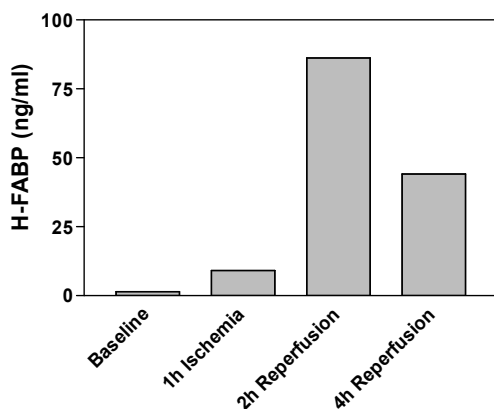
Life Diagnostics, Inc., Catalog Number: 2310-4

Enzyme Immunoassay for the Quantitative Determination of Dog Cardiac Fatty Acid Binding Protein (H-FABP)

INTRODUCTION

Fatty acid-binding proteins (FABP) are cytosolic proteins of about 15 kD. They bind long chain fatty acids and play an important role in fatty acid metabolism. Heart, liver and intestinal FABP isoforms exist. Heart has a high content of FABP (10-20 mol % of cytoplasmic proteins) and heart FABP (H-FABP) has proved to be a sensitive biomarker of myocardial necrosis in humans. H-FABP is rapidly released into the circulation from damaged cardiac muscle. Serum/plasma levels are significantly increased within 1-4 hours of muscle injury and values return to normal within 12 to 24 hours. Because H-FABP is also expressed in skeletal muscle, it is necessary to exclude or control for skeletal muscle injury before ascribing H-FABP elevations to cardiac injury. As shown in the Figure below, H-FABP serves as a useful marker of cardiac injury in dogs.

Plasma H-FABP Levels in a Dog Cardiac Ischemia-Reperfusion Model



PRINCIPLE OF THE TEST

The dog H-FABP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-dog H-FABP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-dog H-FABP antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 30 minutes. This results in H-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The

concentration of H-FABP is proportional to the optical density of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-dog H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard (lyophilized), containing 60 ng/ml dog H-FABP when reconstituted as detailed on the vial label
- 10x Diluent (25 ml)
- 20x Wash Solution (50 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips.
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer.
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an OD range of 0-4 OD
- Graph paper (PC graphing software is optional)

STORAGE OF TEST KIT

The unused kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

All reagents should be allowed to reach room temperature (18-25°C) before use.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The dog H-FABP standard is provided in lyophilized form. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved to obtain a 60 ng/ml stock (**the reconstituted standard should be aliquoted and frozen at -20°C after reconstitution if further use is intended**).
2. Label 8 polypropylene or glass tubes as 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0 ng/ml

3. Dispense 400 μl of diluent into the tube labeled 20 ng/ml and 300 μl of diluent into the remaining tubes.
4. Pipette 200 μl of the 60 ng/ml H-FABP standard into the tube labeled 20 ng/ml and mix. This provides the working 20 ng/ml H-FABP standard.
5. Prepare a 10 ng/ml standard by diluting and mixing 300 μl of the 20 ng/ml standard with 300 μl of diluent in the tube labeled 10 ng/ml. Similarly prepare the 5, 12.5, 2.5, 1.25, 0.625 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: In plasma samples from a dog ischemia-reperfusion model we found that peak H-FABP levels of ~85 ng/ml were achieved 2h after reperfusion. Baseline levels were approximately 1 ng/ml. We suggest that samples initially be tested after a 5-fold dilution in 1x sample diluent.

1. Dispense 240 μl of 1x diluent into separate tubes.
2. Pipette and mix 60 μl of each serum/plasma sample into a tube containing 240 μl of diluent. This provides a 5 fold diluted sample.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μl of standards and samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
4. Remove the incubation mixture by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (350 μl /well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Add 100 μl of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 30 minutes.
9. Wash as detailed in 4 and 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
11. Dispense 100 μl of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
13. Stop the reaction by adding 100 μl of Stop Solution to each well.
14. Gently mix. It is important to make sure that all the blue color changes to yellow.
15. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.

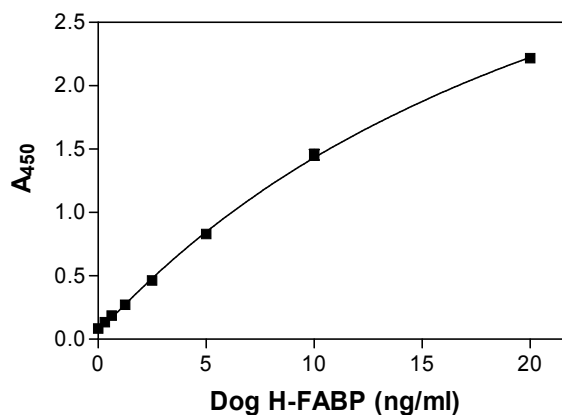
3. Using the mean absorbance value for each sample, determine the corresponding concentration of H-FABP in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of H-FABP in the serum/plasma sample.
5. If available, PC graphing software may be used for the above steps.
6. If the OD_{450} values of samples fall outside the standard curve samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against H-FABP concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns.

H-FABP (ng/ml)	Absorbance (450 nm)
20	2.218
10	1.455
5	0.830
2.5	0.464
1.25	0.272
0.625	0.187
0.3125	0.135
0	0.084

Representative Dog H-FABP Standard Curve



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

RAT H-FABP ELISA KIT (HIGH SENSITIVITY)

Life Diagnostics, Inc., Catalog Number: 2310-2-HS

ELISA for the Quantitative Determination of Rat Cardiac Fatty Acid-Binding Protein (H-FABP) in Serum or Plasma

INTRODUCTION

Fatty acid-binding proteins are cytoplasmic proteins of about 15 kDa that bind long chain fatty acids and play an important role in fatty acid metabolism. Different types of FABP have been detected including Heart FABP (H-FABP), Liver FABP and Intestinal FABP. Human cardiac muscle has a high content of H-FABP (10-20 mol % of cytoplasmic proteins) and H-FABP is a sensitive biomarker of myocardial necrosis that can be used to confirm or exclude a diagnosis of acute myocardial infarction (AMI). In AMI, H-FABP is rapidly released from damaged cardiomyocytes into the circulation due to its solubility and small size. Human clinical studies indicate that H-FABP levels are significantly elevated above threshold within 3 hours of AMI and subsequently return to normal values in 12 to 24 hours. H-FABP has also been identified as a potential serum biomarker for stroke that is superior to either neuron specific enolase or S100B. Our high sensitivity rat H-FABP kit is offered as a tool for investigation of heart damage in rat models of cardiovascular disease¹.

PRINCIPLE OF THE TEST

The H-FABP Quantitative test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses an affinity purified anti-rat H-FABP antibody for solid phase (microtiter wells) immobilization and a horseradish peroxidase (HRP) conjugated anti-rat H-FABP antibody for detection. The test sample is diluted and incubated with conjugate in the microtiter wells for 60 minutes. This results in rat H-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes at room temperature, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of H-FABP is proportional to the optical density.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-rat H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8).
- HRP Conjugate Reagent, 11 ml.
- Reference standard stock (lyophilized)
- 10x Diluent (25 ml)
- Wash Buffer (20x stock, 50 ml)
- TMB Reagent (One-Step) 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes and tips.
- Distilled water.
- Polypropylene microcentrifuge tubes (1.5 ml).
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Plate shaker with an approximate mixing speed of 100 rpm.
- Microtiter plate reader (450 nm wavelength) with an optical density range of 0-4 OD.
- Graph paper (PC graphing software is optional).

STORAGE OF TEST KIT AND INSTRUMENTATION

The reference standard stock provided with the kit should be frozen at or below -20°C on receipt. **The remainder of the kit should be stored at 2-8°C** and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date shown on the kit package, provided that the components are stored as described above.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. It may be necessary to dilute serum samples with the assay diluent in order to obtain values within the standard range.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

WASH BUFFER PREPARATION

The wash buffer is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The rat H-FABP standard is provided as a lyophilized stock. Reconstitute with the volume of diluent indicated on the vial label. Mix gently until the contents of the vial dissolve. **The reconstituted standard should be aliquoted and frozen at or below -20°C after reconstitution if additional use is intended.**
2. Prepare a working 5 ng/ml standard according to the instructions on the reference standard vial label.
3. Label 7 polypropylene or glass tubes as 2.5, 1.25, 0.625, 0.313, 0.156, 0.078 and 0 ng/ml and pipette 250 µl of diluent into each tube.
4. Into the tube labeled 2.5 ng/ml, pipette and mix 250 µl of the 5ng/ml standard. This provides the 2.5 ng/ml standard.

- Similarly prepare the 1.25, 0.625, 0.313, 0.156, 0.078 ng/ml standards by serial dilution.

SAMPLE PREPARATION

Serum or EDTA plasma may be used in the assay. Avoid use of heparin plasma. Baseline levels of rat H-FABP are in the range of 1 – 2 ng/ml and can increase to 30 ng/ml or higher following cardiac injury¹. We recommend that samples be diluted 5-fold prior to assay. This may be achieved by mixing 50 μ l of each test sample with 200 μ l of 1x diluent.

ASSAY PROCEDURE

- Secure the desired number of coated wells in the holder.
- Dispense 100 μ l of standards and samples into the wells (we recommend that samples be tested in duplicate).
- Add 100 μ l of enzyme conjugate reagent into each well.
- Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 60 minutes.
- Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 μ l/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
- Dispense 100 μ l of TMB Reagent into each well.
- Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
- Stop the reaction by adding 100 μ l of Stop Solution to each well.
- Gently mix. *It is important to make sure that all the blue color changes to yellow.*
- Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

CALCULATION OF RESULTS

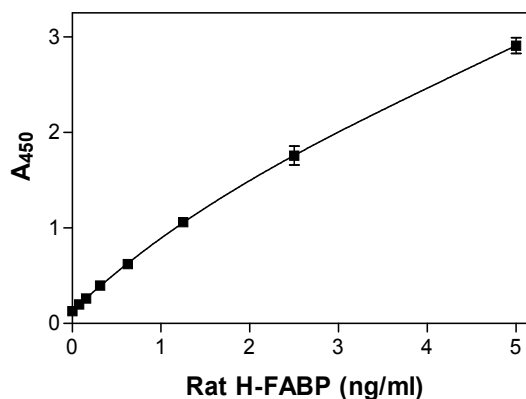
- Calculate the average absorbance values (A_{450}) for each set of reference standards, and samples.
- Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
- Using the mean absorbance value for each sample, determine the corresponding concentration of H-FABP in ng/ml from the standard curve.
- Multiply the derived concentrations by the dilution factor to determine the actual concentration of H-FABP in the sample.
- PC graphing software may be used for the above steps.
- If the OD_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown on the Y axis against H-FABP concentrations shown on the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

H-FABP (ng/ml)	Absorbance (450 nm)
5	2.909
2.5	1.758
1.25	1.059
0.625	0.622
0.313	0.397
0.156	0.261
0.078	0.198
0	0.129

Representative Rat H-FABP Standard Curve



LIMITATIONS OF THE PROCEDURE

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

REFERENCES

- Yoshitani K, Qing M, Zhu B, Burkhard Mackensen G and Podgoreanu MV. Strain-Specific Differences in Sensitivity to Myocardial Injury after Cardioplegic Arrest in the Rat. Am. Soc. Anesthesiol., Annual Meeting Abstracts, Oct. 15 2007, A1326

PIG CK-MM ELISA KIT

Life Diagnostics, Inc., Catalog Number: 2112-3

ELISA for the Determination of Pig Creatine Kinase MM (CK-MM) in Serum or Plasma

- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- Plate reader with an optical density range of 0-4 at 450nm
- Graph paper (PC graphing software is optional).

INTRODUCTION

Creatine kinase (CK) is a key metabolic enzyme. It is a dimer of two subunits, each with molecular weights of approximately 43 kDa. Two different subunits occur, M and B. The CK holoenzyme therefore exists as MM and BB homodimers and an MB heterodimer. The MM and BB isoforms are expressed primarily in skeletal muscle and brain respectively while both MB and MM are expressed in heart. The relatively high expression levels of the MB isoform in heart explain its use as an established biomarker for heart disease. Likewise, CK-MM can be used as a specific biomarker for skeletal muscle injury. Studies in pigs have demonstrated that serum CK-MM levels can increase 50-fold or more as a result of skeletal muscle injury (ref. 1).

PRINCIPLE OF THE TEST

The pig CK-MM ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified CK-MM antibodies for solid phase immobilization and a horseradish peroxidase (HRP) conjugated CK-MM monoclonal antibody for detection. Standards and diluted test samples are incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. As a result, CK-MM molecules are sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of CK-MM is proportional to the optical density of the test sample and actual concentrations are determined by reference to a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-pig CK-MM antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- 2x HRP Conjugate, 6 ml
- Reference pig CK-MM stock (lyophilized)
- Wash Buffer (20x stock, 50 ml)
- Diluent (50 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer

STORAGE

The kit should be stored at 2-8°C and the microtiter strips should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase provided that the components are stored as described.

GENERAL INSTRUCTIONS

1. Please read the entire kit insert before starting the ELISA.
2. All reagents should be allowed to reach room temperature (18-25°C) before use.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

HRP CONJUGATE PREPARATION

The HRP conjugate is provided as a 2x stock. Prior to use estimate the final volume of HRP conjugate required for your assay and dilute one (1) volume of the 2x stock with one (1) volume of diluent. Typically, we prepare 1 ml of conjugate for each 8-well strip used in the assay. This is prepared by mixing 0.5 ml of 2x HRP conjugate with 0.5 ml of diluent. Mix gently prior to use. Prepare the working conjugate no more than one hour in advance.

STANDARD PREPARATION

1. The reference CK-MM stock is provided in lyophilized form. Reconstitute with diluent as directed on the vial label and mix gently until dissolved (**the reconstituted standard remains stable for 1 day at 2-8°C**).
2. Label 5 polypropylene or glass tubes as 50, 25, 12.5, 6.25 and 3.13 ng/ml.
3. Prepare the 50 ng/ml standard as described on the reference stock vial label.
4. Dispense 250 µl of diluent into the tubes labeled 25, 12.5, 6.25 and 3.13 ng/ml.
5. Pipette 250 µl of the 50 ng/ml CK-MM standard into the tube labeled 25 ng/ml and mix. This provides the working 25 ng/ml CK-MM standard.
6. Similarly prepare the 12.5, 6.25 and 3.13 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: Samples must be diluted at least 10-fold with the provided diluent before use. Sample dilutions ≥ 10 -fold eliminate interference caused by serum/plasma components. In validation studies we observed baseline plasma CK-MM levels of ~200 ng/ml.

However, CK-MM levels may vary depending on experimental conditions and optimum sample dilutions should be determined empirically. Assuming that a dilution of 10-fold is used; samples should be treated as follows:

1. Pipette and mix 25 µl of the serum/plasma sample with 225 µl of diluent in a polypropylene micro centrifuge tube. This provides a 10 fold diluted sample in sufficient volume for duplicate determinations.
2. Repeat this procedure for each sample to be tested

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
4. Remove the incubation mixture using either a plate washer or by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 5 times with 1x wash solution. This should preferentially be performed using a plate washer (400 µl/well). If a plate washer is not available use a squirt bottle. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Add 100 µl of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
9. Wash as detailed in 4 to 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
11. Dispense 100 µl of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
13. Stop the reaction by adding 100 µl of Stop Solution to each well.
14. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
15. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CK-MM in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of CK-MM in the serum/plasma sample.
5. PC graphing software may be used for the above steps. A linear fit of the data usually works well. However if there is a

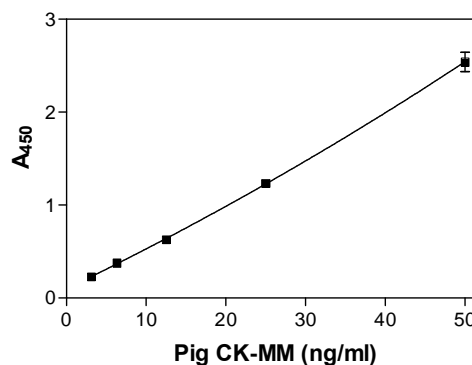
slight curvature of the standard curve, fitting to a polynomial second order equation is advised.

6. If the A_{450} values of samples exceed that of the high standard the samples should be further diluted and re-tested. Samples with absorbance values below the lowest standard should be assigned a value of $<3.13 \times A$ ng/ml, where A represents the dilution factor used for the sample.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against CK-MM concentrations on the X axis is shown below. A standard curve should be run for each experiment.

CK-MM (ng/ml)	Absorbance (450 nm)
50	2.540
25	1.234
12.5	0.626
6.25	0.375
3.13	0.229



LIMITATIONS OF THE PROCEDURE

1. Do not use samples at a dilution less than 10-fold (i.e., do not use a dilution of 5-fold). High serum content of the samples can interfere with the ELISA.
2. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

SPECIFICITY

Pig CK-BB and pig CK-MB were not available when this kit was developed and specificity was therefore investigated using purified human CK-MM, CK-MB and CK-BB. Human CK-MM was strongly recognized but CK-BB was not recognized at concentrations up to 10 micrograms/ml. The ELISA was ~400-fold more selective for CK-MM compared to CK-MB.

REFERENCES

1. K Thoren-Tolling and L Jonsson. Creatine Kinase Isoenzymes in Serum of Pigs Having Myocardial and Skeletal Muscle Necrosis. *Can J Comp Med.* 47:207-216 (1983)