LUMINOMETER SETUP

1. Turn the VetBio-1 luminometer on.
2. Place the tubing from injector 1 into the tube containing background reducer. Tube holders are positioned adjacent to the injectors.
3. Place the tube from injector 2 into the tube containing trigger solution.
4. From the Protocol manager on the keypad select “Prime & Wash”, "Prime", then “Start”.
5. Open the luminometer drawer and insert an empty 12 x 75 mm tube.
6. Close the drawer and click “Start” again.
7. Wait for priming to complete, open the drawer and discard the tube.
8. From the Protocol manager select “Measure” followed by “Cow SAA3”.
9. Select “Start”.
10. The experiment setup screen will be displayed, allowing you to enter experiment name, comments, and the number of samples. After entering the information select “Start”.
11. Enter the sample IDs and dilution factor(s), if different than the default dilution factor.
12. The luminometer is now ready for use.
13. Press “Start” when you are ready to measure luminescence.

REPLICATES

The VetBio-1 luminometer protocols are configured to run singlets of the blank, standard and samples. At the discretion of the user it can be configured to run replicates.

1. From the protocol manager select “Measure” and the program you would like to modify.
2. Select “New” and “Copy Protocol”.
3. Increase the Replicates as desired.
4. Create a new protocol name.
5. Select “Protocols” and save the new protocol.

SAMPLE PREP

This assay was designed specifically for measurement of SAA3 in milk. In milk samples with SCC in the range of 0.06 to 1.1 x 10^6 we found SAA3 levels ranging from 0.5 to 6 μg/ml. Levels as high as 40 μg/ml were found in milk from cows with severe mastitis. We suggest testing milk of normal appearance at a dilution of 100-fold, but optimum dilutions must be determined empirically. To avoid matrix effects do not test milk at dilutions less than 50-fold. A 100-fold dilution can be obtained by mixing 5.0 μl of milk with 0.495 ml of CSD50-1 diluent.

STANDARD PREP

Prepare the 100 ng/ml standard by diluting 10 μl of the SAA3 stock with 0.99 ml of CSD50-1 diluent.

CONJUGATE PREP

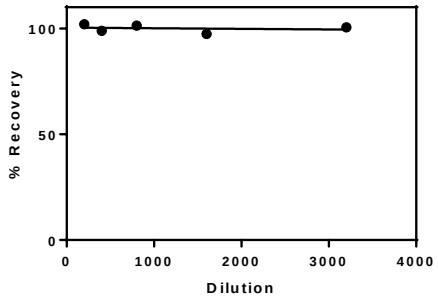
1. The acridan and HRP conjugate mix should be prepared just before use (step 5 in the Procedure section).
2. Tap the vial to ensure that the contents are at the bottom of the vial before carefully removing the stopper.
3. Add 5.5 ml of diluent CSD50-1 to the vial. Insert the stopper and mix gently by inversion several times.
4. Each vial of reconstituted conjugate mix provides enough reagent to measure 0 and 100 ng/ml standards and up to eight samples.[3]

PROCEDURE

1. Determine the number of 12 x 75 mm borosilicate glass tubes required for the assay. Ensure that all tubes fit easily in the VetBio-1 sample holder.
2. Pipet 100 μl of diluent into assay tube one. This serves as the zero standard.
3. Pipet 100 μl of the 100 ng/ml SAA3 standard into tube two.
4. Pipet 100 μl aliquots of the diluted samples into tubes 3, 4, 5… as defined by your assay format.
5. Add 0.50 ml of freshly prepared conjugate mix to each tube and mix gently. A vortex mixer may be used if available.
6. Incubate the mixtures at room temperature.
7. After 45 minutes insert tube 1 into the sample holder of the luminometer and close the drawer. The luminometer automatically injects 50 μl of background reducer and 100 μl of trigger solution, then measures luminescence (RLU/s).
8. Once the RLU/s value is recorded on the screen open the drawer and discard the tube.
9. Determine luminescence for the remaining tubes.
10. SAA3 concentrations are automatically calculated.
11. After measurement of the last sample, select “End”.
12. Results will be saved but may be exported as Excel or pdf files via a USB stick.

[3] If using multiple vials of conjugates to measure more than eight samples, combine the reconstituted contents of all vials and mix briefly before dispensing 0.5 ml aliquots into the reaction tubes. Larger volumes of background reducer and trigger solution must also be used.

Linearity: To assess the linearity of the assay, a milk sample containing SAA at a concentration of 6.1 μg/ml was serially diluted with diluent CSD50-1 to produce values within the dynamic range of the assay.



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For technical assistance please email us at info@vetbiomarkers.com