FavorPrep™ Urine DNA Extraction HE Mini Kit

■ Kit Contents

Cat. No.	FAUD1030 (4 Preps)	FAUD1033 (50 Preps)	FAUD1034 (100 Preps)
FAUD Buffer	3 ml	30 ml	60 ml
W1 Buffer (Concentrate)▲	0.8 ml x 2	12 ml	24 ml
Elution Buffer	0.5 ml	5 ml	7 ml
Lysozyme (Lyophilized) ■	3 mg	20 mg	36 mg
Lyticase Solution	100 µl	550 µl x 2	550 µl x 4
Proteinase K (Liquid)	100 µl	1050 µl	1050 µl x 2
FAUD HE Columns	4 pcs	50 pcs	50 pcs x 2
Collection Tubes	8 pcs	100 pcs	100 pcs x 2
Elution Tubes	4 pcs	50 pcs	100 pcs
User Manual	1	1	1
Preparation of **W1 Buffer** and **Lysozyme**			
Volume of Ethanol for W1 Buffer▲	1 ml	18 ml	36 ml
Volume of sterile ddH_2O for Lysozyme ■	75 µl	500 µl	900 µl

1. All kit components are shipped at room temperature and should be stored at 15~25°C upon receipt, **except Lysozyme** and **Lyticase Solution**.
2. Store Lysozyme and Lyticase Solution at -20°C upon receipt.

■ Specification

Format/Principle	Spin column (Silica matrix)
Binding Capacity	≤125 µg DNA/Column
Operation Time	<45 mins
Sample Size	300 µl Urine
Elution Volume	30 µl

■ Procedure Overview

Urine Sample

- Add 300 µl Urine, 8 µl Lysozyme, 20 µl Lyticase Solution into a tube.
- Incubate at 37°C for 10 mins.
- Add 20 µl Proteinase K and 500 µl FAUD Buffer at 60°C for 10 mins.

- Stand at room temperature for 3 mins.
- (Optional) Add RNase A at room temperature for 2 mins.
- Add 150 µl ethanol (96~100%).

FAUD HE Column

Centrifuge
12,000 xg, 1 min

- Transfer the mixture into the FAUD HE Column for DNA binding.

Centrifuge
12,000 xg, 1 min

- Add 500 µl W1 Buffer (ethanol contained).
- Add 900 µl ethanol (96~100%).

Centrifuge
12,000 xg, 2 mins

- Dry the column membrane.

Centrifuge
12,000 xg, 1 min

- Add 30 µl Elution Buffer.
- Stand the column for 5 mins at room temperature.
- Obtain purified genomic DNA.

■ Preparation Before Starting

1. Add the indicated volume of sterile ddH_2O to the Lysozyme tube and vortex the solution to ensure complete dissolution as a 40 mg/ml stock solution. **Aliquot the Lysozyme stock into small portions and store at -20˚C.**
2. Add the indicated volume of ethanol (96~100%) into the **W1 Buffer**, mix well, and store at room temperature.
3. Additional materials: 96~100% ethanol, RNase A.
4. Set up two water baths or dry baths: one at 37˚C for the **Lysozyme** & **Lyticase** incubation step; the other at 60˚C for **Proteinase K** incubation and **Elution Buffer** preheating.

■ General Protocol

1. Add 300 µl urine sample, 8 µl **Lysozyme** and 20 µl **Lyticase Solution** into an eppendorf (not provided). Mix thoroughly.
   - For DNA isolation from Gram-positive bacteria, urine sample requires an additional incubation at 95˚C for 5 mins before lysozyme and lyticase reaction.
2. Incubate the mixture at 37˚C for 10 mins to disrupt cell wall.
3. Add 20 µl **Proteinase K** and 500 µl **FAUD Buffer** into the sample mixture. Mix thoroughly.
   - **DO NOT** add Proteinase K directly into FAUD Buffer.
4. Incubate the mixture at 60˚C for 10 mins to lyse the sample. During incubation, vortex the tube every 5 mins.
5. Incubate the sample mixture at room temperature for 3 mins.

   (Optional) If RNA-free genomic DNA is required, add 12 µl of RNase A (50 mg/ml; not provided). Mix thoroughly and incubate at room temperature for 2 mins.
6. Add 150 µl ethanol (96~100%) to the sample mixture. Mix gently by pipetting or inverting.
7. Placed a **FAUD HE Column** in a **Collection Tube**, then carefully transfer all mixture into the FAUD HE Column.
8. Centrifuge at 12,000 xg for 1 min. Discard the flow-through and place the FAUD HE Column in a new Collection Tube.
9. Add 500 µl **W1 Buffer** (ethanol contained) to the FAUD HE Column. Centrifuge at 12,000 xg for 1 min then discard the flow-through.
10. Add 900 µl ethanol (96~100%) to the FAUD HE Column. Centrifuge at 12,000 xg for 1 min then discard the flow-through.

General Protocol

11. Centrifuge at 12,000 xg for 2 mins to dry the membrane. Discard the flow-through and the Collection Tube.
12. Place the FAUD HE Column in an **Elution Tube**, then add 30 µl prewarmed **Elution Buffer** or ddH_2O (pH 7.5~9.0) directly onto the membrane. Stand the FAUD HE Column for 5 mins.
    - **Important step!** For effective elution, ensure that the elution solution is dispensed onto the membrane center and absorbed completely.
13. Centrifuge at 12,000 xg for 1 min to elute the DNA.