FavorPrep™ Milk Bacterial DNA Extraction Kit

Cat. No.: FAMBD 000 (4 preps)
FAMBD 001 (50 preps)
(For Research Use Only)

Kit Contents:

	FAMBD 000 (4 preps)	FAMBD 001 (50 preps)
Lysis Buffer MB1	2 ml	25 ml
Lysis Buffer MB2	2 ml	30 ml
W1 Buffer (Concentrate)*	1.3 ml	22 ml
Wash Buffer (Concentrate)**	1 ml	15 ml
Elution Buffer	1 ml	8 ml
Lysozyme ■	3 mg	36 mg
Proteinase K (Liquid)	100 µl × 2	1050 µl × 2
Binding Column W4	4 pcs	50 pcs
Collection Tube	4 pcs	50 pcs
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■ Store lyophilized Lysozyme at -20°C upon receipt of kit.

Preparation of W1 Buffer and Wash Buffer and **store at RT.**		
Cat. No.	FAMBD 000	FAMBD 0001
Ethanol volume for W1 Buffer *	0.5 ml	8 ml
Ethanol volume for Wash Buffer **	4 ml	60 ml

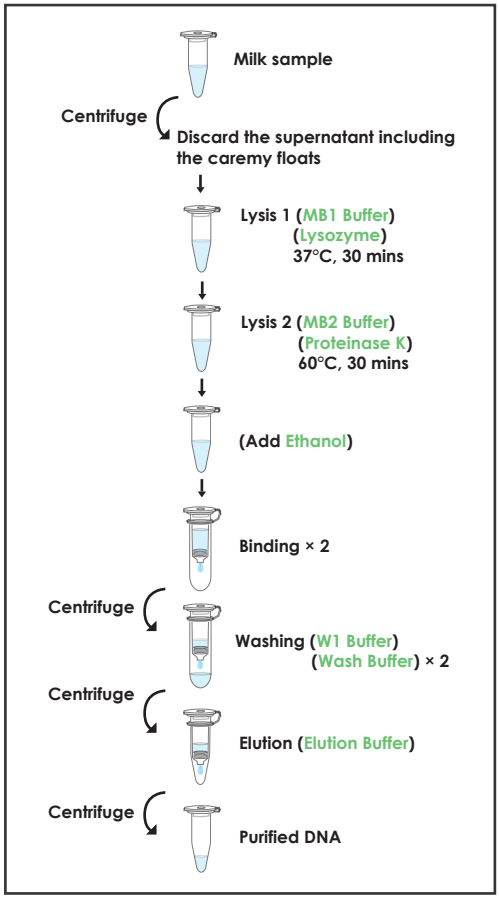
Specification:

Format/Principle: Mini spin column (silica matrix)
Sample Size: Up to 1 ml milk
Operation Time: <75 mins
Binding Capacity: ≤60 µg/column
Column Applicability: Centrifugation

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Add **75 µl** (FAMBD 000) or **0.9 ml** (FAMBD 001) sterile ddH2O to lysozyme tube to make a **40 mg/ml** stock solution. Vortex and make sure that lysozyme has been completely dissolved. **Aliquot the lysozyme stock into small fractions and store the unused portions at -20°C.**
3. Add required volume ethanol (96~100%) to W1 Buffer and Wash Buffer at the first use.
4. Prepare two dry baths or two water baths before the operation: one to 37°C for step 2 and the other to 60°C for step 3.
5. Perheat the Elution Buffer or ddH2O for step 11 (Elution step).
6. All centrifuge steps are done at full speed (14,000 rpm or 18,000 x g) in a microcentrifuge.

Brief procedure



General Protocol:

Please Read Important Notes Before Starting the Following steps.

Hint: Perheat the Elution Buffer or ddH_2O for step 11 (Elution step).

1. Transfer **up to 1 ml of milk sample** to a microcentrifuge tube (not provided) and centrifuge at full speed for 3 mins. Discard the supernatant including the creamy floats on the top layer after centrifugation and use a paper towel or a cotton swap to remove any white remains on the tube wall.
2. Add **425 µl Lysis Buffer MB1 and 15 µl Lysozyme solution (40 mg/ml)** and mix well by vortexing. Incubate at 37°C for 30 mins.
3. Add **425 µl Lysis Buffer MB2 and 40 µl Proteinase K** to the sample mixture and mix thoroughly by vortexing. Incubate at 60°C for 30~60 mins.
4. Add **450 µl ethanol (96~100%)** to the sample mixture. Mix thoroughly by pulse-vortexing for 10 secs.
5. Place a Binding Column W4 to a Collection Tube. Transfer the sample mixture **up to 750 µl** to Binding Column W4 and centrifuge at full speed for 1 min. Discard the flow-through and place the Binding Column W4 back to the Collection Tube.
6. Repeat Step 5 for the rest of the sample mixture. Place the Binding Column W4 to a new Collection Tube.
7. **Add 400 µl W1 Buffer** to Binding Column W4 and centrifuge at full speed for 30 secs. Discard the flow-through and place the Bindibg Column W4 back to the Collection Tube.
   -Make sure that ethanol has been added into W1 Buffer at the first use.
8. **Add 650 µl Wash Buffer** to Binding Column W4 and centrifuge at full speed for 30 secs. Discard the flow-through and place the Binding Column W4 back to the Collection Tube.
   -Make sure that ethanol has been added into Wash Buffer at the first use.
9. Repeat Step 8 for one more washing.
10. Centrifuge at full speed for an additional 3 mins to dry the Binding Column W4 completely.
11. Place Binding Column W4 to a Elution Tube. Add 50~100 µl of preheated Elution Buffer or ddH_2O (pH 7.5-9.0) to the membrane center of Binding Column W4. Stand the Binding Column W4 for 3 mins.
    -Note! Make sure that the Elution Buffer is dispensed onto the membrane and is absorbed completely.
12. Centrifuge at full speed for 1 min to elute total DNA. Store the extracted DNA at 4°C or -20°C.