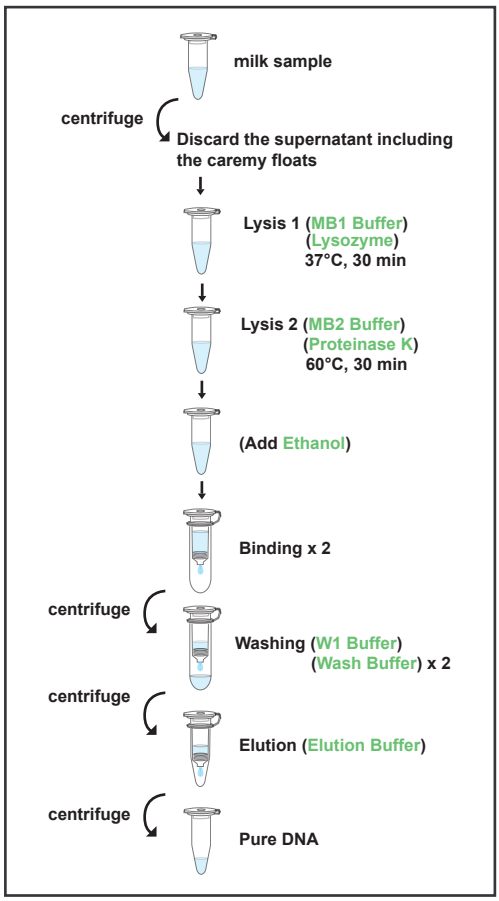
FavorPrep™ Milk Bacterial DNA Extraction Sample Kit

(Cat.: FAMBD 000-Mini, 4 preps)
(For Research Use Only)

Kit Contents:

Lysis Buffer MB1	2 ml
Lysis Buffer MB2	2 ml
W1 Buffer (concentrate)*	1.3 ml
Wash Buffer (concentrate)**	1.0 ml
Elution Buffer	1 ml
Lysozyme ■	3 mg
Proteinase K ■■	1 mg
Binding Column W4	4 pcs
Collection Tube	4 pcs

*Add 0.5 ml ethanol (96-100%) to W1 Buffer
**Add 4 ml ethanol (96-100%) to Wash Buffer
■ Store lyophilized Lysozyme at -20 °C upon receipt of kit
■■ Store lyophilized proteinase k at 4 °C upon receipt of kit



Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Add 150 µl sterile ddH2O to lysozyme tube to make a **20 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 100 µl sterile ddH2O to Proteinase K tube to make a **20 mg/ml** stock solution. Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4 °C.**
4. Add required volume ethanol (96- 100 %) to Wash Buffer W1 and W2 when first use.
5. 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.
6. Perheat the Elution Buffer or ddH2O for step 11 (Elution step).
7. All centrifuge steps are done at full speed (14,000 rpm or 18,000 x g) in a microcentrifuge.

General Protocol:

Please Read Important Notes Before Starting The Following steps.

Hint: Perheat the Elution Buffer or ddH2O 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 minutes. 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 MB1and 30 µl Lysozyme solution (20mg/ml)** and mix well by vortexing. Incubate at 37°C for 30 minutes.
3. Add **425 µl Lysis Buffer MB2 and 20 µl Proteinase K solution (20mg/ml)** to the sample mixture and mix thoroughly by vortexing. Incubate at 60°C for 30 ~60 minutes.
4. Add **450 µl ethanol (96~100%)** to the sample mixture. Mix thoroughly by pulse-vortexing for 10 seconds.
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 seconds. Discard the flow-through and place the Bindibg Column W4 back to the Collection Tube.
   --Make sure that ethanol has been added into Wash Buffer W1 when first use.
8. **Add 650 µl Wash Buffer** to Binding Column W4 and centrifuge at full speed for 30 seconds. 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 W2 when first use.
9. Repeat Step 8 for one more washing.
10. Centrifuge at full speed for an additional 3 min 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 ddH2O (pH 7.5-9.0) to the membrane center of Binding Column W4. Stand the Binding Column W4 for 3 minutes.
    Note! Make sure that the elution solution is dispensed onto the membrane and is absorbed completely.
12. Centrifuge at full speed for 1 minute to elute total DNA. Store total DNA at 4°C or -20°C.