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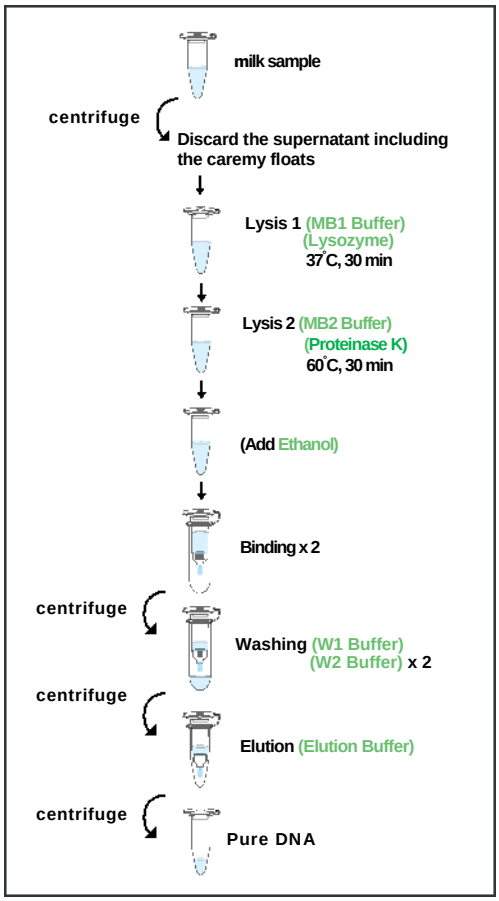
Milk Bacterial DNA Extraction Kit

(Cat.: DE-080 50 preps)

This kit provides a rapid spin column method for the isolation and purification of genomic DNA from both bacteria in milk samples.
Maximum Milk Input: 1 mL

It can also be used to process colostrums milk samples.
Inhibitor-free DNA is ready for PCR, qPCR
Fast and efficient spin-column format



Kit Contents:

Lysis Buffer MB1	**25 ml**
Lysis Buffer MB2	**30 ml**
Wash Buffer W1 (concentrate)*	**22 ml**
Wash Buffer W2 (concentrate)**	**15 ml**
Elution Buffer	**8 ml**
Lysozyme ■	**36 mg**
Proteinase K ■■	**22 mg**
Binding Column W4	**50 pcs**
Collection Tube	**50 pcs**

***Add 8 ml ethanol (96-100%) to Wash Buffer W1**
****Add 60 ml ethanol (96-100%) to Wash Buffer W2**
■ 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 1.8 ml 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 1.1 ml 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. Preheat the Elution Buffer or ddH2O for step 11 (Elution step)
7. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.

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General Protocol:

Please Read Important Notes Before Starting The Following steps.

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)** mix well by vortexing. Incubate at 37°C for 30 minutes.
3. Add **425 µl Lysis Buffer MB1 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. Centrifuge 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. And place the Binding Column W4 to a new Collection Tube.
   Add 500 µl Wash Buffer W1 to Binding Column W4. Centrifuge 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.
   Add 650 µl Wash Buffer W2 to Binding Column W4. Centrifuge 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.
7. Repeat Step 8 for one more washing.
8. Centrifuge for an additional 3 min to dry the Binding Column W4 completely.
9. Place Binding Column W4 to Elution Tube. Add 50~100 µl of 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.
10. Centrifuge for 1 minute to elute total DNA. Store total DNA at 4°C or -20°C.

Special Protocol

1. Add 1.8 ml of dd H2O to the test tube containing lysozyme to obtain a concentration of 20 mg / ml. Vortex and make sure the lysozyme is completely dissolved. (aliquot and store at - 20 ° C)

2. Add 1.1 ml of dd H2O to the test tube containing Proteinase K to obtain a concentration of 20 mg / ml. Vortex and make sure the lysozyme is completely dissolved. (aliquot and store at 4 ° C)

3. Add the required volume of ethanol (96-100%) to the Wash Buffer W1 and W2 before use.

4) Transfer 50 ml of colostrum / milk into a Falcon and centrifuge at 2000 g for 20 '. Remove the supernatant with a pipette and remove the surface grease with wood depressor, while also trying to gently clean the tubing walls;

5) Add 425 µl of MB1 Lysis Buffer, vortex and transfer the mixture to a 2 ml Eppendorf tube;

6) Add 30 µL of Lysozyme (20 mg / ml) and vortex briefly. Incubate in thermomixer at 37 ° C for 30 minutes;

7) Add 425 µl of Lysis Buffer MB2 and 20 µl Proteinase K, vortex briefly and incubate at 60 ° C for 30-60 minutes;

8) Add 450 µl of ethanol (96-100%) and vortex for 10 sec;

9) Transfer the sample mixture to an elution columns Binding Column W4, maximum 750 µl, and centrifuge for 1 minute at 10000 g (14000 rpm); Remove the holder and place the same on a new support for columns;

10) repeat step 6 until the sample is finished.

11) Add 500 µl of Wash W1 Buffer and centrifuge 30 sec. To 10000 g, remove the cartridge holder and place the same on a new column support (make sure that the Buffer W1 has been reconstituted with ethanol before use);

12) Add 500 µl of Wash W2 Buffer and centrifuge 30 sec to 10000 g, remove the cartridge holder and place it on a new column support (make sure that the Buffer W2 has been reconstituted with ethanol before use)

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13) Repeat step 9 more than once

14) Centrifuge 3 min to 10000 g to remove traces of ethanol and to completely dry the column

15) Place the column on a 1.5 ml eppendorf tube and add 50-100 µl of Elution Buffer or dd H2O (pH 7.5 to 9.0), incubate at room temperature for 3 minutes (make sure that the elution solution is dispensed on the membrane and is completely adsorbed);

16) Centrifuge for 1 minute to 10000 g to flow the DNA;

17) Repeat step 13 if necessary, otherwise store the DNA caps at 4 ° C for immediate use or at -20 ° C.

SAMPLE	spectrophotometric reading µg/ml	Purity OD 260/ 280	Purity OD 260/ 230	Real Time Ct average
1 only colostrum	39,1	1,08	1,06	24,64
2 only colostrum	9,8	1,77	1,52	28,12
3 colostrum + 15 ml PBS	11,1	1,78	1,34	26,10
4 colostrum + 15 ml PBS	10,3	1,75	1,52	22,98

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