Milk DNA Extraction Kit (Magnetic Beads)

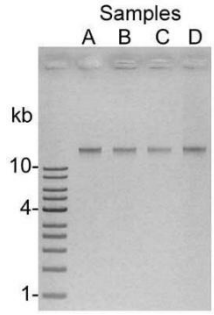
Catalog No.	50024S	50024L
Preps	24 preps	48 preps

Description

The **Milk DNA Extraction Kit (Magnetic Beads)** was developed for the extraction of nucleic acids from various sources in milk, including bacteria (both gram-negative and gram-positive), fungi, yeast, and host cells. It is also suitable for extracting DNA from mastitic milk samples. This magnetic bead-based kit offers a simple and reliable method for DNA extraction from milk samples, eliminating the need for tedious centrifugation steps.

The process begins with enzymatic digestion and lysis buffer, which breaks down bacteria, yeast, fungi, and host cells. Magnetic beads are then added to bind the DNA released from the lysed cells. After the initial DNA separation from the beads, a second bead-binding step is performed to remove additional impurities. Finally, the DNA is washed with ethanol and can be eluted in either elution buffer or TE buffer.

The standard input volume for milk is 500 µl. The yield of extracted DNA may vary depending on several factors, such as the bacterial species, type of milk, and cell density. The extracted DNA is suitable for a wide range of downstream applications, including PCR, qPCR, hybridization, restriction digestion, DNA sequencing, and Next-Generation Sequencing (NGS).



DNA from milk samples were isolated and loaded on a 1% agarose gel. DNA ladder: BioDynami 1 kb DNA Ladder (Cat.# 10004L).

	Specifications
Technology	Magnetic beads
Milk input volume	500 µl
DNA extraction	Bacteria (gram-positive & gram-negative); yeasts; fungi, host cells
Elution volume	> 20 µl
O.D. 260/280	Around 1.8

Features

- High yield of DNA extraction from various milk samples
  - Including mastitic milk samples
- DNA sources from milk
  - Bacteria (gram-negative; gram-positive)
  - Yeasts
  - Fungi
  - Host cells
- Simple procedure
  - No centrifuge needed
  - No column needed
  - No vacuum needed

Component

Catalog No.	50024S	50024L
Milk Lysis Buffer	6 ml	12 ml
MLQ Buffer	5 ml	10 ml
Pre-ISG Buffer	10 ml	20 ml
Milk A Beads	6 ml	12 ml
Milk B Beads	4 ml	8 ml
Lysozyme	0.6 ml	1.2 ml
Proteinase K	0.25 ml	0.5 ml
Elution Buffer	1.2 ml	2.4 ml

Storage Condition

- Store Lysozyme, Proteinase K, Milk A Beads and Milk B Beads at 4°C, stable up to 12 months.
- Store Milk Lysis Buffer, MLQ Buffer, Pre-ISG Buffer, and Elution Buffer at room temperature, stable up to 12 months.

Reagent & Equipment Needed (not provided in this kit)

- Magnetic particle concentrator
- Vortexer
- Heat blocks
- 1.5 ml and 2.0 ml tubes
- Isopropanol (for preparation of ISG Buffer)
- 80% ethanol (prepare before use)

Protocol

Note:

- Resuspend Milk A beads and Milk B beads completely before use.
- Preparation of **ISG Buffer**: Mix 80 µl of Pre-ISG Buffer with 320 µl of isopropanol in a tube or bottle before use. 400 µl of the ISG Buffer is needed per sample. Master mix can be prepared for multiple samples.

1. Add 500 µl of milk samples to 2 ml tubes.
   Optional: add 25 µl of Lysozyme if gram-positive bacteria or unknown strains are present. Add 5 µl of lysostaphin (2 mg/ml, not provided) if lysozyme-resistant gram-positive bacteria are present. Incubate at 37°C for 30 min.
2. Add 10 µl of Proteinase K and 250 µl of Milk Lysis Buffer, mix by vortexing, incubate at 56°C for 30 min.
3. Add 250 µl of the Milk A Beads, mix by vortexing, incubate samples in the thermal mixer at 56°C for 5 minutes with agitation speed at 1000 rpm. Alternatively, incubate samples in a heat block at 56°C for 5 min and vortex the tube from time to time.
4. Put samples on the magnet for 2 min and remove the supernatant.
5. Add 400 µl of the newly prepared ISG Buffer (**see above**), vortex briefly to resuspend beads, incubate for 3 min, vortex briefly to resuspend beads.
6. Put samples on the magnet for 5 min and remove the supernatant.
7. Add 200 µl of the MLQ Buffer, mix by vortexing for 30 sec, incubate at 42°C for 3 min, vortex briefly to resuspend beads, put samples on ice for 5 min.
8. Put samples on the magnet for 2 min, then transfer the supernatant to 1.5 ml tubes.
9. Add 160 µl of the Milk B Beads, mix by vortexing for 30 sec, incubate at 42°C for 5 min. Put samples on the magnet for 2 min and remove the supernatant.
10. Add 800 µl of 80% ethanol, incubate for 2 min. Put samples on the magnet and discard the supernatant. Repeat the 80% ethanol washing one more time.
11. Dry the beads for 8 min and remove any residue solution using a pipette with fine tips. **Please DO NOT over dry the beads. Move to step 12 immediately if beads start to crack**.
12. Add 20-50 µl of Elution Buffer (10 mM Tris, pH 8.0), TE buffer, or Low TE buffer, resuspend the beads completely by pipetting or vortexing, and put samples on ice for 5 min.
13. Put samples on the magnet for 2 min and transfer the supernatant (containing DNA) to new tubes.

Troubleshooting

Low DNA concentration

1. The yield of extracted DNA may vary depending on several factors, such as the bacterial species, type of milk, and cell density.
2. Resuspend Milk A beads and Milk B beads completely before use.
3. Mix completely after adding the Milk Lysis Buffer.
4. Mix completely after adding the Milk A beads and Milk B beads.
5. Make sure the beads are not overdried at step 11.
6. Make sure all beads are resuspended in the Elution Buffer at step 12.

Low O.D. 260/230

1. Discard supernatant completely (steps 4, 6, and 9).
2. Remove any residue solution using a pipette with fine tips at step 11.

Quality Control

Kit components passed stringent functional quality test.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to biodynami.com for Material Safety Data Sheet of the product.

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