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DATA SHEET

Version: 03
Revision date: 21/04/2023

1. Identification

Product name **HigherPurity™ Milk Bacterial DNA Extraction Kit**

Cat. No **AN0242 (50 reactions)**

2. Description

The **HigherPurity™ Milk Bacterial DNA Extraction Kit** is intended for the extraction of bacterial DNA from milk.
The Kit uses a combination of mechanical and chemical lysis to homogenize milk samples. The mechanical disruption ensures complete lysis of Gram-positive and Gram-negative bacteria. After the samples have been homogenized, lysis mixtures should be cleared by centrifugation in order to remove contaminants and residual cellular debris. The clear supernatant is then mixed with the binding buffer to create conditions for optimal binding to the silica membrane column. After washing for efficient removal of potential PCR inhibitors, DNA can be eluted in low salt buffer or water, and is ready-to-use in subsequent reactions

3. Kit Components

Item	Quantity
Minispin columns	50
Collection tubes (2 mL)	100
Bead tubes	50
Lysis Buffer MLK	30 ml
Enhancer Buffer	5 ml
Proteinase K*	30 mg
EC Buffer	8 ml
Binding Buffer	50 ml
WB2 Buffer**	10 ml
EB Buffer	10 ml

*Dissolve Proteinase K in 1.3 ml nuclease-free water to obtain stock solution. The Proteinase K solution can be stored for several days at 2–8 °C. For longer-term storage, the unused portion of the solution may be stored in aliquots at –20 °C until needed. This product as supplied is stable at room temperature.

**Add the volume ethanol (96%-100%) specified [Not included] to WB2 Buffer prior to initial use (see the label on the bottle for a volume indication). After ethanol has been added, mark the bottle to indicate that this step has been completed.

4. Kit Storage

Store the kit at room temperature. If any kit reagent forms a precipitate, warm at 55–65 °C until the precipitated dissolves and allow to cool to room temperature before use.

5. Features

- ✓ Silica membrane technology.
- ✓ Rapid purification of high-quality, ready to use DNA.
- ✓ Complete removal of contaminants and inhibitors for reliable downstream applications.
- ✓ Sample size: up to 250 µl.

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6. Further information

Product Use Limitations	This product is developed, designed, and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Safety Information	When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.canvaxbio.com where you can find, view, and print the MSDS for each CANVAX kit.

PROTOCOL

1. Add **250 µl of milk sample** into a **2.0 mL bead tube** and add **600 µL of Lysis Buffer MLK**. Vortex.
2. Add **100 µL of Enhancer Buffer**. Mix well by shaking gently the microtube.
3. Add **25 µl of Proteinase K**. Incubate at 65°C for 10 minutes.
4. Homogenize by bead beating for 10 minutes at maximum speed on the Vortex Genie 2 or similar using a horizontal adapter.
5. Centrifuge at 14.000 rpm for 5 minutes. Transfer up to 400 µL of the supernatant to a clean microcentrifuge tube.
   IMPORTANT: *A creamy layer floating may be present on top. Avoid transfer of this layer with the supernatant. This layer should be removed in order to prevent clogging of the column*.
6. Add **150 µl EC Buffer**. Vortex. Incubate at 0-4°C for 5 minutes.
7. Centrifuge at 14,000 rpm for 3 minutes. Transfer up to 500 µl of supernatant in a new 1.5 ml microtube avoiding touching the pellet.
8. Add **900 µl of Binding Buffer** and vortex briefly.
9. Assemble a spin column with one of the provided collection tubes. Add 700 µl mixture sample onto the spin column. Close the cap and centrifuge at 8.000 rpm for 1 minute. Discard the flowthrough and reassemble the spin column to a new Collection Tube.
   Repeat step 9 with the remaining sample mixture.
   Ensure that the entire sample mixture has passed into the collection tube by inspecting the column. If sample remains in the column, centrifuge again at 14,000 rpm for 1 minute.
10. Carefully open the spin column and add **700 µl Buffer WB2** (ethanol added). Close the cap and centrifuge at 14.000 rpm for 1 min. Discard the flow-through.
11. Dry silica membrane. Centrifuge at 14.000 rpm for 3 minutes.
12. Place the Column into a 1.5 mL nuclease-free tube (not provided) and add **50-100 µL Pre-heat the EB Buffer at 65°C**. Incubate at room temperature for 2 minutes.
13. Centrifuge at 14.000 rpm for 1 minute, then discard the column. The purified DNA is in the tube.