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Milk Bacterial DNA Isolation Kit — Product Insert

Product #21550

Norgen's Milk Bacterial DNA Isolation Kit is designed for the rapid preparation of genomic DNA from the various bacterial species found within milk. The kit allows for the isolation of genomic DNA from both Gram negative and Gram positive bacteria found in milk samples. The genomic DNA is preferentially purified from other cellular proteinaceous components. Typical yields of genomic DNA will vary depending on the bacterial density of the milk sample, as well as the bacterial species present. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with PCR, quantitative PCR and DNA microarray system.

Norgen's Purification Technology

Purification is based on spin column chromatography. The process for the isolation of genomic DNA from the bacteria found in milk involves first centrifuging the milk sample in order to pellet any bacteria which may be present. If DNA is to be isolated from Gram Positive or unknown strains of bacteria, the pellet is then resuspended in Resuspension Solution A in order to break down the cell wall of the bacteria, followed by the addition of Buffer SK and Proteinase K with an incubation step. If DNA is to be isolated from Gram Negative strains of bacteria, the pellet is then resuspended in Buffer SK. For all types of bacteria, the next step involves the addition of ethanol to the lysate, and the solution is loaded onto a spin column. The spin column binds DNA in a manner that depends on ionic concentrations, thus the DNA will bind to the column while most of the RNA and the digested proteins will flowthough or be retained on the top of the column. The bound DNA is then washed using the provided Buffer SK and Wash Solution A in order to remove any remaining impurities, and the purified bacterial genomic DNA is eluted with Elution Buffer B.

Specifications

Kit Specifications	
Maximum Milk Input	1 mL
Time to Complete 10 Purifications	1 hour
DNA Yield*	500 ng to 8 µg
Bacteria Species Processed	Gram positive and Gram negative
Minimum Detection Limit	10 bacteria in 1 mL of milk

* The range of the DNA yield will vary depending upon a number of factors including bacterial species and type of milk (fat content and %)

Advantages

- Fast and easy processing using a rapid spin-column format
- Isolate genomic DNA from both Gram positive and Gram negative bacteria found in milk
- DNA can be isolated and detected from milk samples with very low bacterial densities (10 bacteria in 1 mL of milk)
- Isolate high quality genomic DNA

Kit Components

Component	Product #21550 (50 samples)
Resuspension Solution A	6 mL
Buffer SK	60 mL
Wash Solution A	18 mL
Elution Buffer B	15 mL
Proteinase K-12mg	12 mg
Lysozyme (powder)	120 mg
Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. The **Lysozyme** should be stored at -20°C upon arrival, and the **Resuspension Solution A** should be stored at -20°C after addition of the lysozyme. The lyophilized **Proteinase K** should be stored at -20°C upon arrival and after reconstitution. These reagents should remain stable for at least 1 year in their unopened containers.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

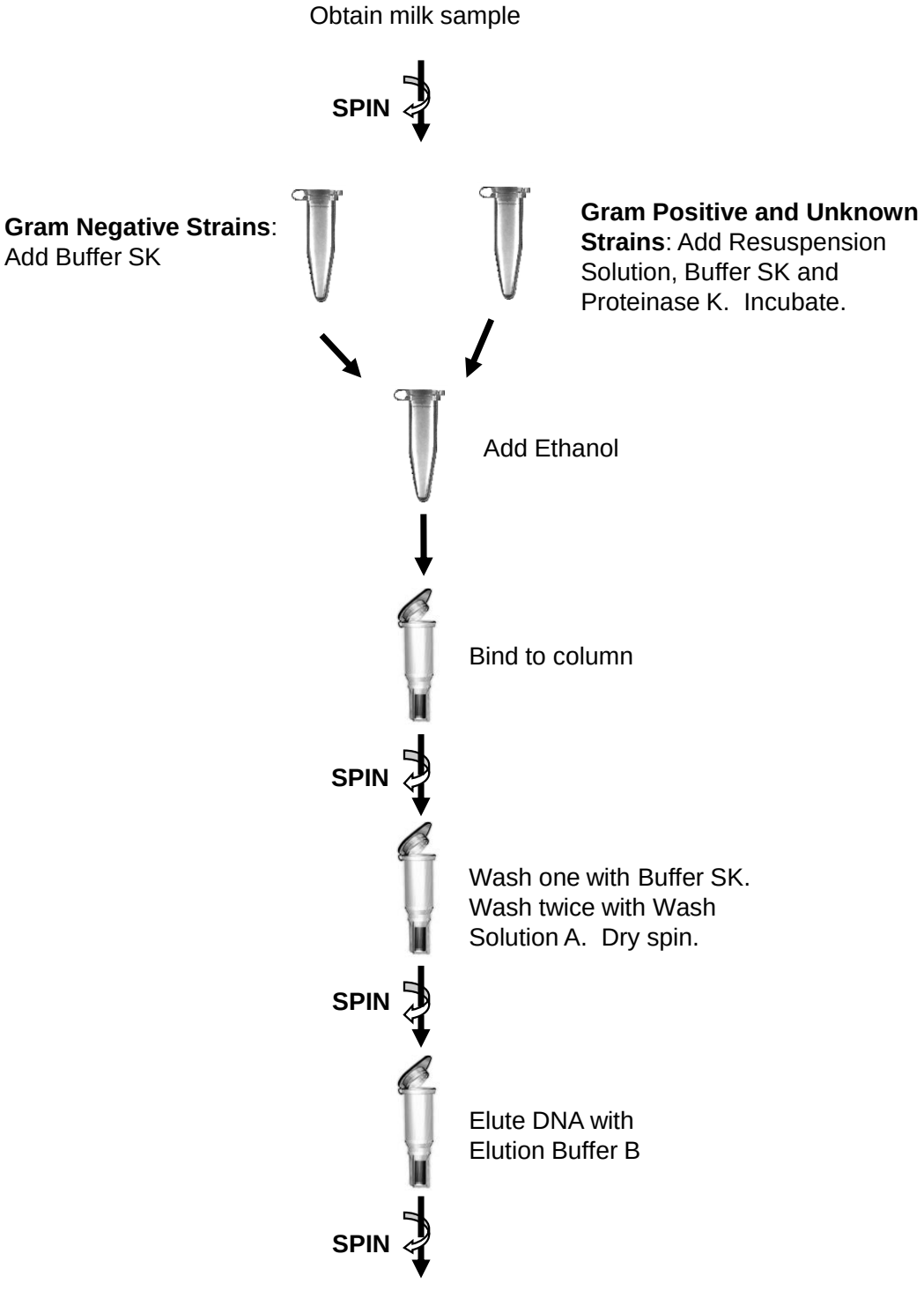
Buffer SK contains guanidine salts, and should be handled with care. Guanidine salt forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Customer-Supplied Reagents and Equipment

- Microcentrifuge tubes
- Benchtop microcentrifuge
- Micropipettors
- 55°C incubator
- 37°C incubator (for Gram positive strains only)
- Lysostaphin (Optional for Gram positive strains only)
- 96 – 100% ethanol
- Cotton swab

Flow Chart

Procedure for Purifying Bacterial Genomic DNA from Milk Samples using Norgen's Milk Bacterial DNA Isolation Kit



Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Notes prior to use:

- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Preheat an incubator or heating block to 55°C (and 37°C for unknown or Gram positive strains).
- Prepare a working concentration of **Wash Solution A** by adding 42 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution A**. This will give a final volume of 60 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- Reconstitute the **Proteinase K** in 600 µL of molecular biology grade water, aliquot into small fractions and store the unused portions at -20°C until needed.
- Add the provided amount of **Resuspension Solution A** to the tube containing the **Lysozyme**, and mix well. Aliquot the **Resuspension Solution A** into small fractions and store the unused portions at -20°C until needed.
- For Gram positive bacteria that are known to be lysozyme-resistant, such as some *Staphylococcus* species, supplement the **Resuspension Solution A** with lysostaphin (not provided). For each isolation, add 5 µL of a lysostphin solution (2 mg/mL in water) to 100 µL of **Resuspension Solution A** (with **Lysozyme**)
- If only Gram negative bacteria are to be isolated, please follow the procedure outlined in Step **1A**. For unknown or Gram positive bacteria, Step **1B** should be performed.

1A. Lysate Preparation (Gram Negative Bacteria)

a. Aliquot a maximum of 1 mL of milk into a microcentrifuge tube.

Note: Up to 1 mL of milk is recommended for normal milk samples or subclinical mastitis samples. For clinical mastitis samples, particularly those with high leukocyte counts, up to 200 µL of milk sample is recommended. If the sample is very viscous and difficult to pipette, pass the sample through an 18-gauge syringe a few times to reduce the viscosity.

b. Centrifuge at 14,000 RPM (~20,000 x g) for 3 minutes.

c. Pour off the supernatant by quickly inverting the tube and gently tapping it against the wall of the waste container. This tapping is to ensure that the creamy layer present on the top of the milk sample after centrifugation is removed. Ensure that the pellet is not dislodged.

d. Add 400 µL of **Buffer SK** and mix well by vortexing.

e. Proceed to Section 2: Sample Binding to Column.

1B. Lysate Preparation (Unknown or Gram Positive Bacteria)

a. Aliquot a maximum of 1 mL of milk into a microcentrifuge tube.

Note: Up to 1 mL of milk is recommended for normal milk samples or subclinical mastitis samples. For clinical mastitis samples, particularly those with high leukocyte counts, up to 200 µL of milk sample is recommended. If the sample is very viscous and difficult to pipette, pass the sample through an 18-gauge syringe a few times to reduce the viscosity.

b. Centrifuge at 14,000 RPM (~20,000 x g) for 2 minutes.

c. Pour off the supernatant by quickly inverting the tube and gently tapping it against the wall of the waste container. This tapping is to ensure that the creamy layer present on the top of the milk sample after centrifugation is removed. Ensure that the pellet is not dislodged.

d. Resuspend the pellet in 100 µL of **Resuspension Solution A** (with **lysozyme**)**.** Incubate at 37°C for 45 minutes. Mix the digestion occasionally by vortexing.

Note: Ensure that the provided lysozyme has been added to the Resuspension Solution A.

e. After incubation, add 300 µL of **Buffer SK** and 10 µL of reconstituted **Proteinase K** to the digestion mixture and mix well by vortexing.

f. Incubate the lysate at 55°C for 45 minutes. Mix the lysate occasionally by vortexing.

g. Proceed to Section 2: Sample Binding to Column.

2. Sample Binding to Column

a. Add 200 µL of 96-100% ethanol to the lysis mixture, and mix by vortexing.

b. Assemble a column with one of the provided collection tubes.

c. Using a pipette, carefully transfer the lysate mixed with ethanol to the spin column.

d. Centrifuge the column assembly for 2 minutes at 14,000 RPM (~20,000 x g) to bind the bacterial DNA.

Note: If all the liquid does not pass through the column, spin for an additional 2 minutes at 14,000 RPM (~20,000 x g). If a small amount of liquid still remains on the top the column, proceed to Step **3a** with the addition of **Buffer SK**.

3. Column Wash

a. Apply 500 µL of **Buffer SK** to the column and centrifuge for 2 minutes at 14,000 RPM (~20,000 x g).

b. Discard the flowthrough and reassemble the column and the collection tube.

c. Apply 500 µL of **Wash Solution A** to the column and centrifuge for 1 minute at 14,000 RPM (~20,000 x g).

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

d. Discard the flowthrough and reassemble the spin column with its collection tube.

e. Apply 500 µL of **Wash Solution A** to the column and centrifuge for 1 minute at 14,000 RPM (~20,000 x g).

f. Discard the flowthrough and reassemble the spin column with its collection tube.

g. Centrifuge for 2 minutes at 14,000 RPM (~20,000 x g) to ensure the column is completely dry.

h. Discard the collection tube.

4. DNA Elution

a. Transfer the spin column to a provided 1.7 mL Elution tube.

b. Apply 100 µL of **Elution Buffer B** to the column and centrifuge at 2,000 RPM (~425 x g) for 2 minutes.

c. Spin for an additional 1 minute at 14,000 RPM (~20,000 x g) to complete the DNA elution.

Note: For a more concentrated sample, 50 µL **Elution Buffer B** can be used.

d. The purified DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at –20°C for long-term storage.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
The mini spin column is clogged	The sample is too large	Ensure that no more than 1 mL of subclinical mastitis milk or no more than 200 µL of clinical mastitis milk be used for the procedure in order to prevent clogging of the column. Clogging can be alleviated by increasing the g-force and/or centrifuging for a longer period of time until the lysate passes through the column.
	The milk sample is very viscous	If the sample is very viscous and difficult to pipette, pass the sample through an 18-gauge syringe a few times to reduce the viscosity prior to the first 3 minute spin.
	White, creamy layer was not removed after initial spin	After the first 3 minute spin, ensure that the white, creamy layer floating on top of the milk sample is removed. This layer should be removed in order to prevent clogging of the column.
The lysate is very gelatinous prior to loading onto the column	White, creamy layer was not removed after initial spin	After the first 3 minute spin, ensure that the white, creamy layer floating on top of the milk sample is removed. This layer should be removed in order to minimize the thickness of the lysate.
	The sample is too large	Too many cells are in the lysate preparation. Ensure that no more than 1 mL of milk is used for the procedure. If the milk sample is known to have a high bacterial content or categorized as clinical mastitis, it is recommended no more than 200 µL is used for the input.

Problem	Possible Cause	Solution and Explanation
The yield of genomic DNA is low	The milk sample may not contain any bacterial species	If the milk sample does not contain any bacteria then no genomic DNA will be detected.
	Incomplete lysis of cells	Extend the incubation time of Proteinase K digestion or reduce the amount of milk used for the input. Increase the lysozyme incubation time for Gram positive strains.
	The DNA elution is incomplete	Ensure that centrifugation at 14,000 x RPM is performed after the 2,000 x RPM centrifugation cycle, to ensure that all the DNA is eluted.
The genomic DNA is sheared	The genomic DNA was handled improperly	Pipetting steps should be handled as gently as possible. Reduce vortexing times during mixing steps (no more than 10-15 seconds).

Related Products	Product #
Bacterial Genomic DNA Isolation Kit	17900
Fungi / Yeast Genomic DNA Isolation Kit	27300
Blood DNA Isolation Mini Kit	46300
Food DNA Isolation Kit	54500

Technical Support
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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