

Milk DNA Preservation and Isolation Kit

Product Insert

Product #44800

Norgen's Milk DNA Preservation and Isolation Kit provides a rapid all-in-one procedure for the preservation and isolation of milk DNA at ambient temperatures.

INTENDED USE

Norgen's Milk DNA Preservation and Isolation Kit is an all-in-one solution designed for 1) preservation of DNA in milk samples at ambient temperature; and 2) isolation of high quality DNA within a laboratory setting. The kit is provided with a Preservation Solution that allows for either immediate processing of milk samples, or allows for the storage of the milk samples for up to 1 month at room temperature prior to DNA isolation. Genomic DNA extracted from somatic cells and bacteria found in milk can be used in various applications. The isolated DNA can be used for the detection of biomarkers to diagnose a disease, follow the diseases progress or monitor the effects of a particular treatment. Milk DNA can also be used to diagnose particular types of infections. Milk DNA purified using Norgen's kit is of the highest quality, and is compatible with a number of downstream applications including PCR, Southern Blot analysis, sequencing and microarray analysis.

PRESERVATION SOLUTION

Norgen's Preservation Solution is an aqueous storage buffer designed for rapid cellular lysis and subsequent preservation of DNA from fresh specimens. The preservative prevents the growth of Gram-negative and Gram-positive bacteria and fungi, and also inactivates viruses allowing the resulting non-infectious samples to be handled and shipped safely. In addition, the preservative eliminates the need to immediately process or freeze samples and allows the samples to be shipped to centralized testing facilities at ambient temperature. The components of the preservative allow samples to be stored for 1 month without any detectable DNA degradation.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The genomic DNA is preferentially purified from other cellular components such as proteins and RNA. The process involves first mixing the milk sample with the provided Preservation Solution (please see the flow chart on page 3). The resulting milk sample can be processed immediately, or alternatively can be stored at room temperature for up to 1 month prior to DNA isolation. When DNA isolation is required, Proteinase K and a Purification Additive are then added to the sample, followed by the addition of isopropanol. The resulting solution is spun down, and Buffer SK is then added to the supernatant and the sample is loaded onto a spin-column. Norgen's resin binds DNA in a manner that depends on ionic concentrations. Thus only the DNA will bind to the column, while most of the RNA and proteins will be removed in the flowthrough. The bound DNA is then washed twice with the provided Wash Solution A in order to remove any remaining impurities, and the purified total DNA is eluted with Elution Buffer B. The purified DNA is of the highest quality and can be used in a number of downstream applications.

Specifications

Kit Specifications	
Number of Preps	25
Maximum Milk Input	0.5 mL
Time to Complete 10 Purifications	80 minutes (20 minutes hands on)

Advantages

- Milk samples are stable for 1 month at room temperature (or 1 week at 37°C) in the Preservation Solution
- Fast and easy processing using a rapid spin-column format
- DNA can be isolated and detected from as little as 100 µL of milk
- Isolate high quality genomic DNA

Kit Components

Component	Product #44800 (25 samples)
Preservation Solution E	30 mL
Proteinase K	0.6 mL
Purification Additive	0.5 mL
Buffer SK	40 mL
Wash Solution A	18 mL
Elution Buffer B	8 mL
Mini Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
Product Insert	1

Storage Conditions and Product Stability

The Proteinase K should be stored at -20°C upon arrival and after reconstitution. All other solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

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.

The **Buffer SK** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Milk of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with milk.

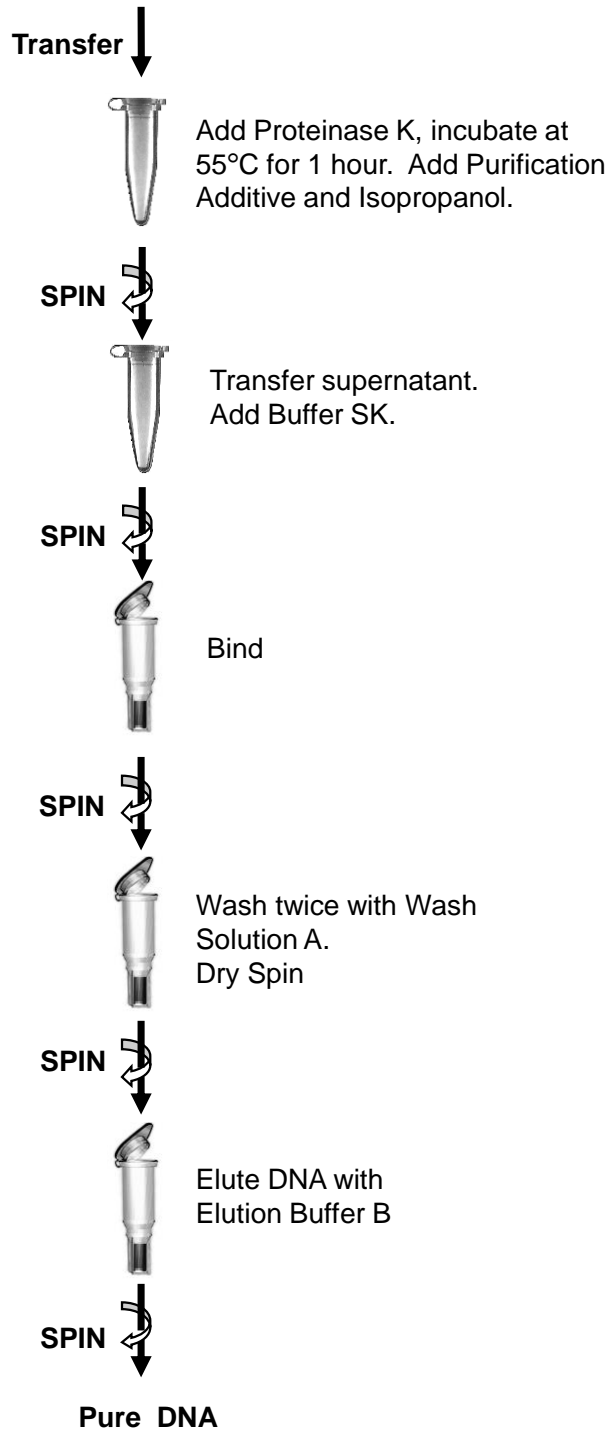
Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- Sterile collection tube
- 96 - 100% ethanol
- Isopropanol
- 55°C incubator
- Optional: Lysozyme (20 mg/mL) and 37°C incubator (for Gram positive bacteria)

Flow Chart

Procedure for Purifying milk DNA using Norgen's Milk DNA Preservation and Isolation Kit

Collect milk sample into vials containing **Preservation Solution E (1:1)**



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
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Freshly collected milk samples are recommended for the use of this kit. Frozen milk may be used as a starting material, however the quality and the yield of the isolated DNA may be lower than if fresh milk is used.
- After milk samples have been mixed with the **Preservation Solution E**, they may be stored at room temperature for up to 1 month, or frozen at -20°C for longer-term storage.
- Ensure that the **Preservation Solution E** is added to all milk samples that will be processed with the kit, including milk samples that will be processed immediately.
- 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.
- Optional: For the isolation of DNA from Gram positive bacteria in the milk prepare a 20 mg/mL stock solution of lysozyme.
- The maximum recommended volume of milk input is 500 µL.

1. Milk Sample Collection and Lysis

Note: The Milk Sample Collection and Lysis procedure is written for an input of 250 µL of milk (500 µL of Preserved Milk). Volumes of up to 500 µL of milk (1 mL of preserved milk) can be processed; please adjust the volumes of the other solutions used in Section 1 accordingly.

- a. Add **Preservation Solution E** in a 1:1 ratio to the milk sample (Example; add 250 µL of Milk DNA Preservation Solution E to 250 µL of milk sample). Mix by vortexing.

Note: At this point, the milk samples may be stored at room temperature for storage up to 1 month, or frozen at -20°C for longer-term storage.

- b. Vortex the preserved milk sample for 10 seconds. Transfer the 500 µL of preserved milk to a sterile microcentrifuge tube (or a 15cc tube if processing 1 mL of preserved milk).
- c. Add 10 µL of **Proteinase K**. Mix by vortexing and incubate at 55°C for 1 hour.

Note: To isolate gDNA from Gram positive bacteria, add 12 µL of previously prepared lysozyme stock solution, mix well and incubate at 37°C for 2 hours (Incubation times may fluctuate between 0.5 and 2 hours depending on the bacterial strain being lysed).

- d. Add 6 μL of **Purification Additive** and mix by vortexing for a few seconds.

Note: Ensure the viscous **Purification Additive** is completely dissolved in the milk mixture.

- e. Add 60 μL of isopropanol and mix by vortexing for a few seconds.
- f. Proceed to Step 2, Sample Binding to Column.

2. Sample Binding to Column

- a. Add 600 μL of the **Buffer SK** to the lysate, mix by vortexing for few seconds.
- b. Assemble a spin column with a provided collection tube. Apply 600 μL of the lysate to the column and centrifuge for 1 minute at 8,000 x g (~8,000 RPM).

Note: Ensure the entire sample has passed through into the collection tube by inspecting the column. If the entire sample volume has not passed, spin for additional 1 minute.

- c. Discard the flowthrough and reassemble the spin column with its collection tube.
- d. Apply another 600 μL of the lysate to the column and centrifuge for 1 minute at 8,000 x g (~8,000 RPM).
- e. Discard the flowthrough and reassemble the spin column with its collection tube.

Note: Each spin column provided is capable of processing up to 1 mL of preserved milk. Repeat steps **d** and **e** to bind the entire volume.

3. Column Wash

- a. Apply 500 μL of **Wash Solution A** to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

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.

- b. Wash column a second time by adding another 500 μL of **Wash Solution A** and centrifuging for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

4. DNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 100 μL of **Elution Buffer B** to the column.
- c. Centrifuge for 2 minutes at **200 x g (~2,000 RPM)**, followed by a 2 minute spin at **14,000 x g (~14,000 RPM)**.

Note: For maximum DNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 4b** and **4c**).

5. Storage of DNA

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.	Centrifugation speed was too low or spin time was inadequate.	Check the centrifuge to ensure that it is capable of generating the required RPMs. Sufficient centrifugal force is required to move the liquid phase through the resin. Also ensure that the correct spin times are followed. Spinning for a few additional minutes will help.
	The sample is too large	Too many cells were applied to the column. Ensure that no more than 1 mL of preserved milk is applied to the column. Clogging can be alleviated by centrifuging for a longer period of time until the lysate passes through the column.
	The lysate/ Buffer SK mixture is not homogeneous	To ensure a homogeneous solution, vortex for 10-15 seconds before applying the lysate to the spin column.
The yield of genomic DNA is low	Incomplete lysis of cells	Increased Proteinase K incubation time at 55°C may result in increased yields
	The DNA elution is incomplete	Ensure that centrifugation at 14,000 x g is performed after the 200 x g centrifugation cycle, to ensure that all the DNA is eluted.
	DNA concentration in the saliva sample being used is low.	Some milk samples contain very little DNA. This varies from individual to individual based on numerous variables. Increased proteinase K incubation time at 55°C may result in increased yields may result in increased yields.
	Preservation Solution E was not added to the milk samples.	Preservation Solution E must be added to the milk samples even if they are not to be stored for long term.
DNA is degraded	Preservation Solution E was not completely mixed with the milk sample.	Ensure Preservation Solution E was mixed well with the saliva sample.
DNA does not perform well in downstream applications.	DNA was not washed two times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed two times with the Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
RNA is present in eluted DNA.	RNA is coeluted with the DNA.	Carry out a digestion with RNase A on the elution if the RNase present will interfere with downstream applications. Refer to manufacturer's instructions regarding amount of enzyme to use, optimal incubation time and temperature.

Related Products	Product #
Milk Bacterial DNA Isolation Kit	21550
Saliva DNA Collection, Preservation and Isolation Kit	RU35700
2X PCR Master Mix	28007
Taq DNA Polymerase with 10X Taq Reaction Buffer Plus	28089

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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