A&A BIOTECHNOLOGY
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Manual

Genomic Mini AX Milk Spin

Increased efficiency kit for genomic DNA purification from milk.

catalog #	size
059-100S	100 isolations

For research use only.

Guarantee
A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Table of Contents

Contents

component	100 isolations	storage
Mini AX Spin columns	100 pcs	2–8 °C
2 ml tubes	200 pcs	15–25 °C
LSU lysis buffer	50 ml	15–25 °C
W1 first wash solution	70 ml	15–25 °C
W2 second wash solution	60 ml	15–25 °C
E elution buffer (without EDTA)	20 ml	2–8 °C
N neutralizing buffer	1 ml	15–25 °C
T solution	400 µl	2–8 °C
Proteinase K	2 x 1.1 ml	2–8 °C

The binding capacity of the column is 15 µg.

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf tubes
- 15 ml Falcon tubes
- PBS buffer or TE buffer (cat. # K-TE-5, K-TE-100)
- Incubator or thermoblock set to 50 °C
- Vortex
- Microcentrifuge, centrifuge with refrigerated swing-out rotor

Optional

- RNAse (cat. # 1006-10, 1006-50)

Important information

- E elution buffer loses activity upon prolonged contact with air. Always close the E elution buffer vial tightly directly after use. Store E elution buffer at 2-8 °C.

Material preparation

1. Transfer **5-10 ml** of milk sample to 15 ml Falcon tube (not included).
2. Centrifuge for **10 min** at **5000 RPM (4500 x g)**.

   During centrifugation a cream is collected at the top of the tube. It creates a kind of plug which firmly adheres to the wall of the tube. After centrifugation we recommend to circle around the edge of the tube with a tip to facilitate removal of the plug.
3. Discard the supernatant.
4. Add **10 ml** of **PBS** buffer or **TE** buffer (not included).
5. Centrifuge for **10 min** at **5000 RPM (4500 x g)**.

   The white pellet is visible at the bottom of the tube.
6. Discard the supernatant.
7. Add **500-600 µl** of **PBS** buffer or **TE** buffer (not included).

   The volume of PBR buffer or TE buffer can be increased according to the obtained pellet. However, the total sample volume can't exceed 1,5 ml.
8. Transfer the sample to **a new 1,5 ml** Eppendorf tube (not included).
9. Centrifuge for **5 min** at **8000 RPM (6000 x g)**.
10. Discard the supernatant.
11. Follow point 1. of the isolation protocol.

Isolation protocol

1. Add **400 µl** of **LSU** lysis buffer and **20 µl** of **proteinase K**.

2. Vortex the sample and incubate for **60 min** at **50 °C**.

   Vortex the sample a few times

   The incubation step can be performed in Eppendorf Thermomixer or analogous equipment at 1400 RPM and 50 °C.

   RNA digestion (optional): add 5 µl of RNAse (10 mg/ml solution) (not included). Mix and incubate for 5 min at room temp.

3. Intensively vortex the sample for **2 min** at **1000-1400 RPM**.

   This is the key step for efficiency of DNA isolation.

4. Centrifuge for **5 min** at **8 000 x g**.

   The DNA pellet should be visible at the bottom of the tube.

5. Apply the sample onto the Mini AX Spin column placed inside a 2 ml tube.

6. Centrifuge for **30-60 s** at **8 000 x g**.

7. Transfer the Mini AX Spin column to **a new 2 ml** tube (included).

8. Add **600 µl** of **W1** first wash solution.
   Centrifuge for **30-60 s** at **8 000 x g**. Discard supernatant.

9. Place the Mini AX Spin column into **the same 2 ml** tube (included).

10. Add **600 µl** of **W1** first wash solution.
    Centrifuge for **30-60 s** at **8 000 x g**.

11. Transfer the Mini AX Spin column to **a new 2 ml** tube (included).

12. Add **500 µl** of **W2** second wash solution.
    Centrifuge for **30-60 s** at **14 000-21 000 x g**.

13. Prepare a 1.5 ml elution tube (not included) and add **5 µl** of **N** neutralizing buffer.

    DNA neutralization - page 6.

14. Transfer the Mini AX Spin column to the prepared elution tube.

15. Before using E buffer, it is recommended to do a functionality test - page 6.

Apply **100-150 µl** of **E** elution buffer onto the Mini AX Spin column.
Keep for **2 min** at **room temp.**

E elution buffer loses activity upon prolonged contact with air. Always close the E elution buffer vial tightly directly after use. Store E elution buffer at 2-8 °C.

16. Centrifuge for **30-60 s** at **14 000-21 000 x g**.

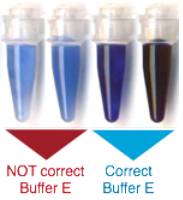
17. Remove the Mini AX Spin column. Close the tube with purified DNA.

DNA neutralization

E elution buffer is strongly alkaline and may cause DNA degradation upon freezing. Thus it is necessary to use a N neutralizing buffer. We recommend adding the N neutralizing buffer to the elution tube before the elution step. If the N neutralizing buffer was not added before the elution step it can be added directly before freezing DNA samples. The use of N neutralizing buffer enables secure DNA storage conditions at 10 mM TrisHCl, pH 8.5.

E buffer functionality test

E buffer has a critical influence on DNA elution efficiency and thus overall DNA purification yield. The kit contains T solution which enables testing of the E buffer correct functionality.



Typically it is suggested to perform such a test in the following cases:
- E buffer was not used for a long period of time (at least 2 months).
- E buffer was stored at room temp. for a long period of time (at least 2 weeks).
- E buffer vial was not closed tightly.

Procedure:
Transfer 20 µl of E buffer to PCR tubes; add 2 µl of T solution; mix the sample, wait 2 min. Compare the mixture color with the reference color guide.

Safety information

DANGER

Proteinase K

H315 Causes skin irritation.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335 May cause respiratory irritation.
P261 Avoid breathing dust.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P342+P311 If experiencing respiratory symptoms call a Poison Center or doctor/physician.

WARNING

LSU lysis buffer

H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

W1 first wash solution

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

E elution buffer

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a Poison Center or doctor/physician.

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