A&A BIOTECHNOLOGY
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Manual

Genomic Mini AX Soil Spin

Increased efficiency kit for genomic DNA purification from soil.

catalog #	size
068-100S	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
R separation solution	60 ml	15–25 °C
BS suspension buffer	15 ml	15–25 °C
LSU lysis buffer	35 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
Lysozyme	1.1 ml	-20 °C
Proteinase K	2 x 1.1 ml	4–8 °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
- Saline solution (0.9% NaCl solution)
- Incubator or thermoblock set to 37 °C, 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

Separation of cells from soil samples

1. Transfer **0.5 g** of soil sample to 15 ml Falcon tube (not included).
2. Add **saline solution** (0.9% NaCl solution)(not included) up to **2.5 ml** of total volume.
3. Vortex for **30 s,** keep on ice for **5 min**.
4. Prepare a new 15 ml Falcon tube (not included).
   Add **500 µl** of **R** separation solution and keep on ice for **5 min**.
5. The **soil suspension** in the first tube should be taken carefully from above the sand sediment and layered on the surface of the separation solution **R** in the second tube.

   The layer of soil suspension cannot be mixed with the layer of separation solution R.
6. Place the tube into a refrigerated swing-out rotor.
   Centrifuge for **10 min** at **4 500 x g**.
7. After centrifugation, the soil pellet should be firmly bound to the bottom of the tube.
   Transfer **all supernatant** (both layers) to a new 15 ml Falcon tube (not included).
8. Add **saline solution** (0.9% NaCl solution)(not included) up to **10 ml** of total volume.
9. Place the tube into a refrigerated swing-out rotor.
   Centrifuge for **10 min** at **4 500 x g**.
10. Carefully discard the supernatant. Suspend the pellet in **100 µl** of **BS** suspension buffer.
11. Follow point 1. of the isolation protocol.

Isolation protocol

1. Add **10 µl** of **lysozyme**.

2. Mix the sample and incubate for **15 min** at **37 °C**.

3. Add **300 µl** of **LSU** lysis buffer and **20 µl** of **proteinase K**.

4. Vortex the sample and incubate for **10 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.

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

   This is the key step for efficiency of DNA isolation.

6. Centrifuge for **10 s** at **8 000 x g**.

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

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

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

9. Transfer the Mini AX Spin column to **a new 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**.

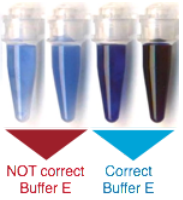
17. Remove the Mini AX Spin column. Close the tube with purified DNA.

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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