

Manual

Genomic Mini AX Tissue 96-well

Increased efficiency kit for genomic DNA purification from tissue and cell culture. Form: 96-well plates.

catalog #	size
056-192	192 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	3
Additional equipment and reagents	3
Necessary	3
Optional	3
Material preparation	4
Solid tissue	4
Cell culture	4
Semen	4
Isolation protocol	4
DNA neutralization	6
E buffer functionality test	6
Safety information	7

Contents

component	192 isolations	storage
P96 purification plate	2 pcs.	4-8 °C
E96 elution plate	2 pcs	room temp.
R96 receiving plate	6 pcs	room temp.
Protective film	10 pcs	room temp.
LSU lysis buffer	90 ml	room temp.
W1 first wash solution	140 ml	room temp.
W2 second wash solution	140 ml	room temp.
E elution buffer	40 ml	4-8 °C
N neutralizing buffer	3 x 1 ml	room temp.
T solution	400 µl	4-8 °C
Proteinase K	4 x 1.1 ml	4-8 °C

Additional equipment and reagents

Necessary

- 1.5 ml / 2 ml sterile Eppendorf tubes
- 1M DTT (cat. # 2010-5, 2010-25, 2010-10P) (for DNA isolation from semen)
- Incubator or thermoblock set to 50 °C
- Centrifuge with swing-out rotor for 96-well plates (8.0 cm high)
- Vortex
- Microcentrifuge

Optional

- RNase (cat. # 1006-10, 1006-50)
- Vorex for 96-well plates
- Self-adhesive foil / protective film

Material preparation

Points 1-4 of isolation protocol can be alternatively performed in a suitable 2 ml 96-deep well plate (not included).

Solid tissue

1. Transfer up to **20 mg of fragmented tissue** to a 1.5 ml / 2 ml tube (not included) or 2 ml 96-deep well plate (not included).
2. Follow point 1. of the isolation protocol.

Cell culture

1. Transfer **1x10⁶ of cell culture** to a 1.5 ml tube (not included) or 2 ml 96-deep well plate (not included).
Close the tube or stick a protective film on the plate (not included).
Centrifuge and discard the supernatant.
2. Follow point 1. of the isolation protocol.

Semen

1. Transfer **100 µl of semen** to a 1.5 ml tube (not included) or 2 ml 96-deep well plate (not included).
2. Add **20 µl of 1M DTT** (not included).
3. Follow point 1. of the isolation protocol.

Isolation protocol

Points 1-4 of isolation protocol can be alternatively performed in a suitable 2 ml 96-deep well plate (not included).

1. Add **400 µl of LSU lysis buffer** and **20 µl of proteinase K**.
Close the tube or stick a protective film on the plate (not included).
2. Vortex the samples and incubate at 50 °C:

cell culture and semen - **30 min**
solid tissue - **1-2 h** (until complete digestion).

Vortex the samples from time to time.

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 samples for **2 min**.

This is the key step for efficiency of DNA isolation.

4. Centrifuge for **5 min** at **10 000 RPM** (tubes) or **10 min** at **2000 xg** (plate).

5. Remove the protective film from the **P96** purification plate.
Assemble the **P96** purification plate with a **R96** receiving plate.

6. Apply the supernatant onto the wells of the **P96** purification plate.
Stick a **new protective film** on the **P96** purification plate.

7. Transfer the assembled plates to the swing-out rotor.

Note: If an odd number of plates use the counter-plate for centrifugation.

Centrifuge for **4 min** at **2 000 x g**.

8. Carefully separate the plates. Remove the **R96** receiving plate.
Assemble the **P96** purification plate with a **new R96** receiving plate. Remove **the protective film** from the **P96** purification plate.

9. Add **600 µl** of **W1** first wash solution. Stick a **new protective film** on the **P96** purification plate.

10. Transfer the assembled plates to the swing-out rotor.

Note: If an odd number of plates use the counter-plate for centrifugation.

Centrifuge for **4 min** at **2 000 x g**.

11. Carefully separate the plates. Remove the **R96** receiving plate.
Assemble the **P96** purification plate with a **new R96** receiving plate. Remove **the protective film** from the **P96** purification plate.

12. Apply **600 µl** of **W2** second wash solution. Stick a **new protective film** on the **P96** purification plate.

13. Transfer the assembled plates to the swing-out rotor.

Note: If an odd number of plates use the counter-plate for centrifugation.

Centrifuge for **4 min** at **2 000 x g**.

14. Prepare a **E96** elution plate and add **5 µl** of **N** neutralizing buffer directly onto each well.

DNA neutralization - page 6.

15. Carefully separate the plates. Remove the **R96** receiving plate. Assemble the **P96** purification plate with the **E96** elution plate.

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

Remove the protective film from the **P96** purification plate.
Apply **150 µl** of **E** elution buffer onto the well of the **P96** purification plate.

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 4-8 °C.

Stick a **new protective film** on the **P96** purification plate.

17. Keep for **5 min** at **room temp.**

18. Transfer the assembled plates to the swing-out rotor.

Note: If an odd number of plates use the counter-plate for centrifugation.

Centrifuge for **4 min** at **2 000 x g**.

19. Carefully separate the plates. Remove the **P96** purification plate.
Stick a **new protective film** of the **E96** elution plate. Store the purified DNA at 4 °C until later use.

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

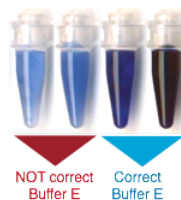
E buffer has a critical influence on DNA elution efficiency and thus overall DNA purification yield. The kit contains T solution which enables testing of the E buffer correct functionality.

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.



WARNING

W1 first wash solution

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

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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