A&A BIOTECHNOLOGY
innovating life science

Manual

Genomic Mini AX Body Fluids

Increased efficiency kit for genomic DNA purification from body fluids.
Procedure with DNA precipitation.

catalog #	size
052-20M	20 isolations
052-40M	40 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	052-20M	052-40M	storage
Genomic Mini AX columns	20 pcs	40 pcs	2–8 °C
15 ml tubes	20 pcs	40 pcs	15–25 °C
2 ml tubes	20 pcs	40 pcs	15–25 °C
L1.4 lysis solution	45 ml	90 ml	15–25 °C
K1 equilibrating solution	20 ml	40 ml	15–25 °C
K2 wash solution	70 ml	135 ml	15–25 °C
K3 elution solution	25 ml	50 ml	15–25 °C
PM precipitation mix	20 ml	40 ml	15–25 °C
TE buffer	5 ml	16 ml	15–25 °C
Proteinase K	1.1 ml	2 x 1.1 ml	4–8 °C

The binding capacity of the minicolumn is 20 µg.

Additional equipment and reagents

Necessary

- 15 ml Falcon Tubes
- 70% ethanol
- Incubator or thermoblock set to 50 °C
- Centrifuge

Important notes

- The chromatography purification of DNA can be paused at any time while a sample is loaded onto a column. The purification process can be continued after a 15-hours-long pause with no influence on quality or quantity of purified DNA. During the pause of the DNA purification the 15 ml tube with the column inside has to be closed with the screw cap to avoid membrane desiccation and subsequent DNA loss.

Protocol

1. Transfer up to **2 ml** of **body fluids sample** (e.g. plasma, urine, amniotic fluid) to a 15 ml Falcon tube (not included).

2. Add **1 volume** of **L1.4** lysis solution (e.g. 2 ml of sample - 2 ml of L1.4 lysis solution) and **50 µl** of **proteinase K**.

3. Mix and incubate for **15 min** at **50 °C**. Mix the samples by inverting the tubes a few times.

4. During incubation prepare the Genomic Mini AX columns placed inside 15 ml tubes. Apply **800 µl** of **K1** equilibrating solution. Wait for the solution to flow through the column.

5. Apply the sample onto the equilibrated Genomic Mini AX column. Wait for the lysate to flow through the column.

   The Genomic Mini AX column works by means of gravity. The flow rate strongly depends directly on the quantity and size of DNA molecules in a sample. High content of high molecular weight DNA decreases the flow rate. DNA amount exceeding 20 µg loaded onto a column may lead to flow stoppage. In such cases the column should be centrifuged in a swing-out rotor for 1 min at 3000-4000 RPM. The centrifugation can be performed after the loading step (point 5) and during the washing step with K2 solution (point 7 and 8).

6. Transfer the Genomic Mini AX column to a 15 ml **tube** (included).

7. Add **1.5 ml** of **K2** wash solution. Wait for the solution to flow through the column.

8. Add again **1.5 ml** of **K2** wash solution. Wait for the solution to flow through the column.

9. Add **100 µl** of **K3** elution solution. Wait for the eluate to flow through the column.

   Note. The purpose of this step is to decrease the total volume of eluate, since the column void volume is about 100 µl.

10. Transfer the Genomic Mini AX column to a **2 ml** precipitation tube (included).

    Note: The column drop director possesses proper fitting that allows easy attachment to the precipitation tube.

11. Add **1 ml** of **K3** elution solution. Wait for the eluate to flow through the column.
Remove the Genomic Mini AX column.

12. PM precipitation mix contains a precipitation enhancer and it should be intensively mixed before use by vigorous hand shaking.

Add **800 µl** of **PM** precipitation mix to the eluted DNA.

13. Mix the sample by inverting the tube a few times and centrifuge for **10 min** at **10 000 RPM (~10 000 x g)**.

The light-blue DNA pellet should be visible at the bottom of the precipitation tube.

14. Carefully discard supernatant. Be careful not to remove the DNA pellet at the bottom of the tube.

Attention. When pouring out the supernatant, it is very easy to lose the DNA pellet. For safety, it is recommended to pour the supernatant into the prepared tube so the pellet can be recovered.

15. Add **500 µl** of **70% ethanol** (not included).
Mix the sample and centrifuge for **3 min** at **10 000 RPM (~10 000 x g)**.

Note. The light-blue DNA pellet should be visible at the bottom of the precipitation tube.

16. Carefully discard supernatant. Be careful not to remove the DNA pellet at the bottom of the tube.

Attention. When pouring out the supernatant, it is very easy to lose the DNA pellet. For safety, it is recommended to pour the supernatant into the prepared tube so the pellet can be recovered.

17. Air dry the plasmid DNA pellet for **5 min** at **room temp**. up-site down.

Note. If there are any leftovers (small droplets) of alcohol on the tube walls they should be removed with sterile cotton buds.

18. Dried DNA pellets can be dissolved in the desired volume of TE buffer.

Note. The blue color of DNA precipitate enables visual confirmation of the DNA dissolution process.

19. Store the plasmid DNA at -20 °C until later use.

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

L1.4 lysis 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.

WARNING

K1 equilibrating 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

K2 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

K3 elution 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

PM precipitation mix

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.

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