A&A BIOTECHNOLOGY
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Manual

Genomic Mini AX Bacteria+

Increased efficiency kit for genomic DNA purification from gram-positive bacteria
Procedure with DNA precipitation.

catalog #	size
060-60M	60 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	060-60M	storage
Genomic Mini AX columns	60 pcs	2–8 °C
2 ml tubes	60 pcs	15–25 °C
BS suspension buffer	7 ml	2–8 °C
LS lysis suspension	60 ml	15–25 °C
K1 equilibrating solution	55 ml	15–25 °C
K2 wash solution	190 ml	15–25 °C
K3 elution solution	70 ml	15–25 °C
PM precipitation mix	55 ml	15–25 °C
Tris buffer (10 mM Tris-HCl, pH 8,5)	10 ml	15–25 °C
Lysozyme	1.3 ml	-20 °C
Mutanolysin	350 µl	-20 °C
Proteinase K	1.3 ml	2–8 °C

The binding capacity of the minicolumn is 20 µg.

Additional equipment and reagents

Necessary

- 1.5 ml, 2 ml Eppendorf tubes
- 70% ethanol
- Incubator or thermoblock set to 50 °C
- Vortex
- Microcentrifuge

Optional

- RNAse (cat. # 1006-10, 1006-50)
- Sterile water (cat.# 003-075, 003-25)
- TE buffer (cat.# 297-100)

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. Centrifuge **0.2-1 ml** of **overnight bacterial culture**. Discard the supernatant.
Suspend the bacterial pellet in **100 µl** of **BS** suspension buffer.

2. Add **20 µl** of **lysozyme** and **5 µl** of **mutanolysin**.

   Recombinant mutanolysin and lysozyme activity is synergistic. Using these mixtures leads to increase yield of bacteria lysis (e.g. *Streptococcus*, *Lactobacillus*, *Lactococcus*, *Listeria*).

   Mix and incubate for **20 min** at **50 °C**.

3. LS lysis suspension should be mixed by inverting the tubes before use.

   Add **900 µl** of **LS** lysis suspension and **20 µl** of **proteinase K**.

4. Vortex the sample and incubate for **10 min** at **50 °C**. Mix the samples by inverting the tubes 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) (not included). Mix and incubate for 5 min at room temp.

5. 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.

6. Vortex the sample for **15 s**.
Centrifuge for **5 min** at **10 000-14 000 RPM**.

   The pellet should be visible at the bottom of the tube. It is a mixture of non-lysed fragments of sample material and particles of the LS lysis suspension.

7. Apply the supernatant 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 7) and during the washing step with K2 solution (point 8 and 9) or elution step with K3 solution (point 10).

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

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

10 Apply **100 µl** of **K3** elution solution directly to the Genomic Mini AX column membrane.
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.

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

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

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

13. 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.

14. Mix the sample by inverting the tube a few times and centrifuge for **10 min** at **10 000 RPM**.

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

15. 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.

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

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

17. 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.

18. 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.

19. Dried DNA pellets can be dissolved in the desired volume of **Tris** buffer (included), **TE** buffer or sterile water (not included).

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

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

Additional information

Pause in the purification process. 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.
The volume of solution in the 15 ml tube enables the easy location of the procedure step after which the DNA purification process was paused:
~ 0.5 ml - after the column equilibration;
~ 1.5 ml - after the sample loading step;
~ 3 ml - after the first washing step with K2 solution;
~ 4.5 ml - after the second washing step with K2 solution.

Gravity flow of column. 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 and during the washing step with K2 solution or during the elution step with K3 solution.
Subsequently, the DNA elution step should be performed as follows:
Transfer the column to a new 15 ml tube (not included). Add 1 ml of K3 elution solution. Wait 2 min and centrifuge for 1 min at 3000 RPM. Transfer the eluate to a 2 ml tube (included). Follow point 13. of the protocol.

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

LS lysis suspension

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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