A&A BIOTECHNOLOGY
innovating life science

Genomic Mini AX Bacteria+

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

version 0318

60 isolations

Cat. # 060-60M

The binding capacity of the genomic DNA purification column is 20 µg of DNA.

For R&D use only

Kit Contents

Component	Quantity	Store at
Genomic Mini AX columns	60 pcs	+4 to +8 °C
2 ml tubes	60 pcs	Room Temp.
BS suspension buffer	7 ml	+4 to +8 °C
LS lysis suspension	60 ml	Room Temp.
K1 equilibrating solution	55 ml	Room Temp.
K2 wash solution	190 ml	Room Temp.
K3 elution solution	90 ml	Room Temp.
PM precipitation mix	55 ml	Room Temp.
Proteinase K	1.3 ml	+4 to +8 °C
Lysozyme	1.3 ml	-20 °C
Mutanolysin recombinant	350 µl	-20 °C
Tris buffer (10 mM Tris HCl, pH 8.5)	10 ml	Room Temp.

Equipment and materials necessary for DNA isolation that are not included in kit

1. Material for DNA isolation
2. RNAse (cat. # 1006-10, 1006-50) (option)
3. 1.5 ml sterile Eppendorf tubes
4. 70% ethanol
5. Incubator set to 50 °C
6. Vortex
7. Benchtop microcentrifuge

NOTE:
Before you start working, we recommend cleaning the work surface using LabZAP™ product (cat. # 040-500).

A&A Biotechnology provides one year guarantee on this kit
The company does not guarantee correct performance of this kit in the event of:
- not adhering to the supplied protocol
- not recommended use of equipment and materials
- the use of other reagents than recommended or which are not a component of the kit
- the use of expired or improperly stored reagents and columns

Isolation protocol

Note

The chromatography purification of DNA can be paused at any time while sample is loaded onto column. The purification process can be continued after up to 15-hours-long pause with no influence on quality or quantity of purified DNA. During the pause of the DNA purification 15 ml tube with column inside has to be closed with the screw cap to avoid membrane desiccation and subsequent DNA lost. See „Additional information" - page 6.

1. Centrifuge 0.2-1 ml of overnight bacterial culture.
   Discard the supernatant.

2. Suspend the bacterial pellet in 100 µl of BS suspension buffer.
   Add 20 µl of lysozyme and 5 µl of mutanolysin (both included).

   Note:

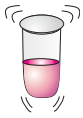
   Recombinant mutanolysin and lysozyme activity is synergistic. Using these mixture leads to increase yield of bacteria lysis (e.g. *Streptococcus, Lactobacillus, Lactococcus, Listeria*).

   Mix and incubate for 20 min at 50 °C.

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

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

4. Mix the samples by vortexing and incubate for 10 min at 50 °C.
   Mix the samples by inverting the tubes a few times.

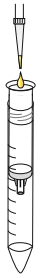


50 °C

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

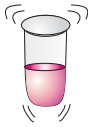
   RNA digestion (optional): See „Additional information" - page 6.

5. During incubation prepare the Genomic Mini AX columns placed inside 15 ml tubes and set the columns with the tubes in the suitable rack.

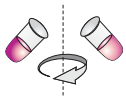


   Apply 800 µl of K1 equilibrating solution onto each column.
   Wait until the K1 equilibrating solution passes through the Genomic Mini AX columns.

6. Mix samples by vortexing for 15 s.



7. Centrifuge for 5 min at 10 000–14 000 RPM.



The DNA 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.

8. Apply the supernatant onto pre-equilibrated Micro AXD columns.

Wait until the lysates pass through the Genomic Mini AX columns.

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 8) and during the washing step with K2 solution (point 9 and 10) or during the elution step with K3 solution (point 11).

See „Additional information" – page 6.

9. Add 1.5 ml of K2 wash solution.
Wait until the K2 wash solution passes through the Genomic Mini AX columns.

10. Add 1.5 ml of K2 wash solution.
Wait until the K2 wash solution passes through the Genomic Mini AX columns.

11. Add 250 µl of K3 elution solution.
Wait until the K3 elution solution passes through the Genomic Mini AX columns.

The purpose of this step is to decrease the total volume of the eluate, since the column void volume is about 250 µl.

12. Transfer the Genomic Mini AX columns to new 2 ml precipitation tubes (included).

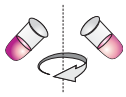
The column drop director possesses proper fitting that allows easy attachment to the precipitation tube.

Add 1 ml of K3 elution solution to the Genomic Mini AX columns. Wait until the K3 elution solution passes through the Genomic Mini AX columns.

13. Remove the Genomic Mini AX columns.

PM solution contains a precipitation enhancer and it should be intensively mixed before use by few times vigorous hand shaking.

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

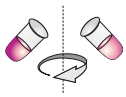


Mix the samples by inverting the tubes a few times and centrifuge for 10 min at 10 000 RPM.

14. Carefully discard supernatants.

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

Add 500 µl of 70% ethanol (not included).



Mix the samples and centrifuge for 3 min at 10 000 RPM.

15. Carefully discard supernatants and air dry the DNA pellets for 5 min at room temp. in the up-side-down position.

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

16. Dried DNA pellet can be dissolved in desired volume of Tris buffer (included), TE buffer (not included) or sterile water (not included).

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

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

Additional Information

Pause in purification process. The chromatography purification of DNA can be paused at any time while sample is loaded onto column. The purification process can be continued after up to
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 lost.

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 equalibration;
~ 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.

RNA digestion. Add 5 µl of RNAse (10 mg/ml solution) (not included, cat. # 1006-10, 1006-50) and mix sample by vigorous vortexing for 20 s.
Incubate the sample for 5 min at room temp.

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 (point 8) and during the washing step with K2 solution (point 9 and 10) or during the elution step with K3 solution (point 11).

Subsequently, the DNA elution step should be performed as follow: Transfer the column to 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 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 vapour.
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 vapours.
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 vapour.
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 vapours.
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 vapour.
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 vapours.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Notes