A&A BIOTECHNOLOGY
innovating life science

Manual

Genomic Mini AX Bacteria 96-well

Increased efficiency kit for genomic DNA purification from bacteria. Form: 96-well plates.

catalog #	size
060-192	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

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.
BS suspension buffer	45 ml	4-8 °C
L1.4 lysis solution	45 ml	room temp.
W1 first wash solution	140 ml	room temp.
W2 second wash solution	140 ml	room temp.
E elution buffer (does not contain EDTA)	40 ml	4-8 °C
N neutralizing buffer	3 x 1 ml	room temp.
T solution	400 µl	4-8 °C
Lysozyme	2 x 1.1 ml	-20 °C
Proteinase K	4 x 1.1 ml	4-8 °C

Additional equipment and reagents

Necessary

- 2 ml sterile Eppendorf tubes or 2 ml 96-deep well plates
- 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 (8.0 cm high)
- Self-adhesive foil / protective film

Material preparation

The material preparation protocol can be alternatively performed in a suitable 2 ml 96-deep well plate (not included).

1. Centrifuge up to **1 ml** of **overnight bacterial culture**. Discard the supernatant.
2. Suspend the bacterial pellet in **200 µl** of **BS** suspension buffer.
3. Add **10 µl** of **lysozyme.** Close the tube or stick a protective film on the plate (not included).

   For *S.aureus* we recommend using lysostaphin (not included, cat # 1007-400, 1007-2000);
   For *Streptococcus*, *Lactobacillus*, *Lactococcus*, *Listeria* z we recommend using mutanolysin recombinant (not included, cat # 1017-5, 1017-10, 1017-50).
4. Incubate for **10 min** at **50 °C**.

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 **200 µl** of **L1.4** lysis solution and **20 µl** of **proteinase K**.
   Close the tube or stick a new protective film on the plate (not included).
2. Vortex the samples and incubate for **10 min** at **50 °C**.
   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 **20 s** at **1000-1400 RPM**.

   This is the key step for efficiency of DNA isolation.
4. Centrifuge for **10 s** at **8 000-14 000 x g** (tubes) or **1 min** at **2000 x g** (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**.

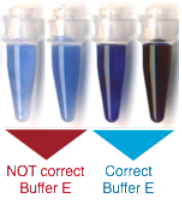
19. Carefully separate the plates. Remove the **P96** purification plate.
Stick **a new protective film** of the **E96** elution plate. Store the purified DNA at 4 °C until later use.

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

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

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.

A&A BIOTECHNOLOGY

innovating life science

A&A Biotechnology, ul. Strzelca 40, 80-299 Gdańsk, Poland
phone +48 883 323 761, +48 600 776 268
info@aabiot.com, www.aabiot.com