A&A BIOTECHNOLOGY
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Manual

Genomic Midi AX Direct

Increased efficiency kit for genomic DNA purification from various sources.
Procedure without DNA precipitation.

catalog #	size
895-20D	20 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	20 isolations	storage
Spin 100AX columns	20 pcs.	2–8 °C
15 ml tubes	40 pcs	15–25 °C
Counterweight cartridge	1 pcs	15–25 °C
L1.4 lysis solution	50 ml	15–25 °C
W1G first wash solution	70 ml	15–25 °C
W2 second wash solution	40 ml	15–25 °C
E elution buffer (without EDTA)	15 ml	2–8 °C
N neutralizing buffer	1 ml	15–25 °C
T solution	400 µl	2–8 °C
TE buffer	50 ml	15–25 °C
Proteinase K	2 x 1.1 ml	4–8 °C

Additional equipment and reagents

Necessary

- 2 ml sterile Eppendorf tubes
- 15 ml sterile Falcon tubes
- Lysostaphin - 15 U/µl (cat. # 1007-3, 1007-15) / Lysozyme - 10 mg/ml (cat. # 1005-10) / Mutanolysin - 10 U/µl (cat. # 1017-5, 1017-10) (for DNA isolation from bacteria)
- Incubator or thermoblock set to 37 °C, 50 °C
- Vortex
- Centrifuge with swing-out rotor
- Microcentrifuge

Optional

- RNAse (cat. # 1006-10, 1006-50)

Material preparation

Fresh or frozen blood samples

1. Transfer **2 ml** of **blood sample** to a 15 ml tube (not included).

   Note: If the sample volume is less than 2 ml add appropriate volume of TE buffer to reach the final volume of 2 ml.

2. Add **2 ml** of **L1.4** lysis solution and **100 µl** of **proteinase K**.

3. Mix the sample by inverting the tube. Incubate for **20 min** at **50 °C**.

   Note: Do not prolong incubation time.

4. Intensely vortex for **20 s**.

5. Follow point 1. of the isolation protocol.

Bacteria (Gram- and Gram+)

1. Transfer **1-5 ml** of **bacterial culture** to a 15 ml tube (not included). Centrifuge and discard the supernatant.

2. Suspend the bacterial pellet in **2 ml** of **TE** buffer.

3. Add **20 µl** of **lysozyme** (10 mg/ml) (not included) and incubate for **15 min** at **37 °C**.

   Note:
   for *S.aureus* we recommend using lysostaphin (15 U/µl) (not included).

   for *Streptococcus*, *Lactobacillus*, *Lactococcus*, *Listeria* we recommend using mutanolysin (10 U /µl) (not included) or **mutanolysin with lysozyme** (not included).

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

4. Add **2 ml** of **L1.4** lysis solution and **100 µl** of **proteinase K**.

5. Mix the sample by inverting the tube. Incubate at **50 °C** until mixture is completely clear (usually 60 min).

   RNA digestion (optional): add 10 µl of RNAse (10 mg/ml solution) (not included). Mix and incubate for 5 min at room temp.

6. Follow point 1. of the isolation protocol.

Cell culture

1. Transfer **1x10^7** of **cell culture** to a 15 ml tube (not included). Centrifuge and discard the supernatant.

2. Suspend the pellet in **2 ml** of **TE** buffer.

3. Add **2 ml** of **L1.4** lysis solution and **100 µl** of **proteinase K**.

4. Mix the sample by inverting the tube. Incubate for **30 min** at **50 °C**.

   RNA digestion (optional): add 10 µl of RNAse (10 mg/ml solution) (not included). Mix and incubate for 5 min at room temp.

5. Centrifuge for **10 min** at **4000-5000 x g**. Transfer the supernatant to a new **1**5 ml tube (not included).
6. Follow point 1. of the isolation protocol.

Fresh tissues

1. Transfer up to **50-100 mg** of **fragmented tissue** or grind in sterile mortar under liquid nitrogen to a 15 ml tube (not included).
2. Add **2 ml** of **TE** buffer, **2 ml** of **L1.4** lysis solution and **100 µl** of **Proteinase K**.
3. Mix the sample by inverting the tube. Incubate at **50 °C** until the tissue will be completely digested (usually 2-4 hours). Vortex the sample from time to time.

   RNA digestion (optional): add 10 µl of RNAse (10 mg/ml solution) (not included). Mix and incubate for 5 min at room temp.
4. Centrifuge for **10 min** at **4000-5000 x g**. Transfer the supernatant to a new **1**5 ml tube (not included).
5. Follow point 1. of the isolation protocol.

Isolation protocol

E lution buffer loses activity upon prolonged contact with air. Always close the tube with E lution buffer vial tightly directly after use.

1. Apply the sample onto the Spin 100AX column placed inside a 15 ml tube.

   Note: If you have an odd number of samples, please remember about counterweight columns before centrifugation.

   Centrifuge in a swing-out rotor for **2 min** at **3000 RPM**.
2. Transfer the Spin 100AX column to **a new** 15 ml tube (included).
3. Add **3 ml** of **W1G** first wash solution. Centrifuge in a swing-out rotor for **2 min** at **3000 RPM**.
4. Add **1.5 ml** of **W2** second wash solution. Centrifuge in a swing-out rotor for **2 min** at **3000 RPM**.
5. During centrifugation, prepare **the new** 15 ml tubes (included) and add **10 µl** of **N** neutralizing buffer to the bottom.

   DNA neutralization - page 6.
6. After centrifugation, transfer the Spin 100AX column to the tube with **N** neutralizing buffer.

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

   Add **400 µl** of **E** elution buffer to Spin 100AX columns.

   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.

8. Keep for **2 min** at **room temp**.

9. Centrifuge for **2 min** at **3000 RPM**.

   In case of an odd number of plates, remember about placing the counterweight plate set before centrifugation.

10. Discard the Spin 100AX column. Transfer eluate to **a new** Eppendorf tube (not included).

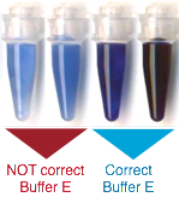
11. Store the DNA at 4-8 °C or -20 °C until later use.

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

W1G 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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