A&A BIOTECHNOLOGY
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Manual

Genomic Midi AX

Increased efficiency kit for genomic DNA purification from various sources.
Procedure with DNA precipitation.

catalog #	size
895-20	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	895-20	storage
Spin 100AX columns	20 pcs	2–8 °C
15 ml tubes	40 pcs	15–25 °C
Counterweight column	1 pcs	15–25 °C
L1.4 lysis solution	45 ml	15–25 °C
K2 wash solution	110 ml	15–25 °C
K3 elution solution	25 ml	15–25 °C
TE buffer	60 ml	15–25 °C
Isopropanol	25 ml	15–25 °C
Proteinase K	2 x 1.1 ml	2–8 °C

The binding capacity of the DNA purification column is 100 µg of DNA.

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf tubes
- 15 ml 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)
- 70% ethanol
- Incubator or thermoblock set to 37 °C, 50 °C
- Vortex
- Centrifuge with swing-out rotor for 15 ml tubes
- Microcentrifuge

Optional

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

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

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 **2.5 ml** of **K2** wash solution. Centrifuge in a swing-out rotor for **2 min** at **3000 RPM**.
4.	Add again **2.5 ml** of **K2** wash solution. Centrifuge in a swing-out rotor for **2 min** at **3000 RPM**.
5.	Transfer the Spin 100AX column to **a new** 15 ml tube (included).
6.	Add **550 µl** of **K3** elution solution.
7.	Incubate for **2 min** at **room temp.** Centrifuge in a swing-out rotor for **1 min** at **3000 RPM**.
8.	Add again **550 µl** of **K3** elution solution. Centrifuge in a swing-out rotor for **1 min** at **3000 RPM**. Remove the Spin 100AX column.

9. The approximate eluate volume should be ~1.1 ml.

   Transfer the eluate to **a new** 2 ml tube (not included).

10. Add **800 µl** of **isopropanol**. Close the tube with a cap and mix carefully by inverting the tube a few times.

    If white precipitate is present in the tubes, follow point A.
    If white precipitate is not present in the tubes, follow point B.

A. The white precipitate is visible

1. Centrifuge for **2 min** przy **4000 RPM**. Carefully discard supernatant.
2. Add **500 µl** of **70% ethanol** (not included).
3. Centrifuge for **2 min** przy **4000 RPM**. Carefully discard supernatant.
4. Air dry the DNA pellet for **10 min** at **room temp.** up-site-down.
5. Dissolve the DNA pellet in the desired volume of **TE** buffer (included) or sterile nuclease-free water (not included).

   To dissolve the DNA easily and completely the sample can be incubated at 50 °C and gently mixed occasionally by subtle shaking.
6. Store the DNA at 4-8 °C or -20 °C until later use.

B. The white precipitate is not visible

1. Transfer the samples to new centrifuge tubes suitable for high centrifuge speed.
   Centrifuge for **15 min** przy **12 000- 14 000 RPM**. Carefully discard supernatant.
2. Add **500 µl** of **70% ethanol** (not included).
3. Centrifuge for **5 min** przy **12 000- 14 000 RPM**. Carefully discard supernatant.
4. Air dry the DNA pellet for **10 min** at **room temp.** up-site-down.
5. Dissolve the DNA pellet in the desired volume of **TE** buffer (included) or sterile nuclease-free water (not included).

   To dissolve the DNA easily and completely the sample can be incubated at 50 °C and gently mixed occasionally by subtle shaking.
6. Store the DNA at 4-8 °C or -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.

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

Isopropanol

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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