A&A BIOTECHNOLOGY
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Manual

Genomic Mini

Kit for genomic DNA purification from various sources.

catalog #	size
116-50	50 isolations
116-250	250 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	116-50	116-250	storage
Minicolumns	50 pcs	250 pcs	15–25 °C
2 ml tubes	50 pcs	250 pcs	15–25 °C
LT lysis solution	13 ml	60 ml	15–25 °C
A1 wash solution	50 ml	250 ml	15–25 °C
Tris buffer (10 mM, pH 8.5)	25 ml	125 ml	15–25 °C
Proteinase K	1.1 ml	5 x 1.1 ml	2–8 °C

Note: if there is precipitation in the LT solution, heat the LT solution up to 40 °C until the precipitate dissolves.

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf 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)
- DTT (cat. # 2010-5, 2010-25, 2010-10P) (for DNA isolation from semen)
- Incubator or thermoblock set to 37 °C, 50 °C, 70 °C
- Vortex
- Microcentrifuge

Optional

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

Material preparation

Bacteria (Gram- and Gram+)

1. Transfer **100 µl** of **bacterial culture** to a 1.5 ml tube (not included).

   Note: For bacterial culture 200 µl-1 ml volume: centrifuge the sample, discard supernatant, suspend the bacterial pellet in 100 µl of Tris buffer.

2. For Gram+ bacteria, we recommend using the following enzymes:

   for *S.aureus* we recommend using lysostaphin (15 U/µl) (not included):
   add **5 µl** of **lysostaphin** and incubate for **10 min** at **37 °C**.

   for *Streptococcus*, *Lactobacillus*, *Lactococcus*, *Listeria* we recommend using mutanolysin (10 U /µl) (not included) or **mutanolysin with lysozyme** (not included):
   add **5 µl** of **mutanolysin** or **5 µl** of **mutanolysin** and **10 µl** of **lysozyme**.
   Mix and incubate for **20 min** at **50 °C**.

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

3. Add **200 µl** of **LT** lysis solution and **20 µl** of **proteinase K**.

4. Mix the whole sample by inverting the tube. Incubate for **20 min** at **37 °C**.

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

5. Follow point 1. of the isolation protocol.

Semen

1. Transfer **100 µl** of **semen** to a 1.5 ml tube (not included).

2. Add **10 µl** of **1M DTT** (not included).

3. Add **200 µl** of **LT** lysis solution and **20 µl** of **proteinase K**.

4. Mix the whole sample by inverting the tube. Incubate for **20 min** at **37 °C**.

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

5. Follow point 1. of the isolation protocol.

Cell culture

1. Transfer **1x10^6** of **cell culture** to a 1.5 ml tube (not included). Centrifuge and discard the supernatant.
2. Suspend the pellet in **100 µl** of **Tris** buffer.
3. Add **200 µl** of **LT** lysis solution and **20 µl** of **proteinase K**.
4. Mix the whole sample by inverting the tube. Incubate for **20 min** at **37 °C**.

   RNA digestion (optional): add 5 µl of RNAse (10 mg/ml solution) (not included). Mix and incubate for 5 min at room temp.
5. Follow point 1. of the isolation protocol.

Fresh tissues

1. Transfer up to **10-15 mg** of **fragmented tissue** to a 1.5 ml tube (not included).
2. Add **100 µl** of **Tris** buffer, **50 µl** of **LT** lysis solution and **20 µl** of **Proteinase K**.
3. Vortex the sample. Incubate at **50 °C** until the tissue will be completely digested. Vortex the sample from time to time.
4. Mix the sample by vigorous vortexing for **20 s**.

   RNA digestion (optional): add 5 µl of RNAse (10 mg/ml solution) (not included). Mix and incubate for 5 min at room temp.
5. Add **150 µl** of **LT** lysis solution and mix the sample.
6. Follow point 1. of the isolation protocol.

Embedded tissues

1. Transfer the **tissue** to a 1.5 ml tube (not included).
2. Add **Tris** buffer, centrifuge and discard the supernatant. Repeat several times to remove the fixing liquid.
3. Remove the paraffin from FFPE samples - rinse in xylene (not included), next rinse in ethanol (not included).

For embedded tissues we recommend Xpure FFPE micro (cat. # 091-50) - kit for genomic DNA purification from tissues preserved in paraffin. Fast deparaffinization without xylene and hexane.

Isolation protocol

Set the thermoblock temperature to 70 °C and place in it the Tris elution buffer (it will be used in point 10. of the isolation protocol).

1. Incubate the sample for **5 min** at **70 °C**.
2. Vortex the samples for **20 s** and centrifuge for **3 min** at **10 000-15 000 RPM**.
3. Apply the supernatants onto the minicolumns. Centrifuge for **1 min** at **10 000-15 000 RPM**.
4. Add **500 µl** of **A1** wash solution.
5. Centrifuge for **1 min** at **10 000-15 000 RPM**.
6. Transfer the minicolumns to **new** 2 ml tubes (included).
7. Add **400 µl** of **A1** wash solution.
8. Centrifuge for **2 min** at **10 000-15 000 RPM**.
9. Transfer the minicolumns to **new** 1.5 ml tubes (included).
10. Add **200 µl** of **Tris** buffer or sterile water (not included) heated to 70 °C directly onto the minicolumn resin.

    Note: If a small amount of DNA is expected then we can reduce the amount of elution buffers (Tris buffer or sterile water) to 100 µl, thus increasing the DNA concentration.
11. Incubate for **2 min** at **room temp.**
12. Centrifuge for **1 min** at **10 000-15 000 RPM**.
13. Remove the minicolumns and store the tube with purified DNA at **4 °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

LT 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

A1 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.

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