A&A BIOTECHNOLOGY
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Manual

Gel-Out

Kit for DNA extraction from agarose.

catalog #	size
023-50	50 isolations
023-250	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	023-50	023-250	storage
Minicolumns	50 pcs	250 pcs	15–25 °C
R7SI agarose melting solution	30 ml	140 ml	15–25 °C
A1 wash solution	50 ml	250 ml	15–25 °C
Sodium acetate (3M, pH 5.5)	500 µl	3 ml	15–25 °C
Isopropanol	15 ml	70 ml	15–25 °C
TE buffer	5 ml	16 ml	15–25 °C

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf tubes
- Incubator or thermoblock set to 50 °C (Eppendorf Thermomixer recommended)
- Vortex
- Microcentrifuge

Additional

- Sterile water (cat.# 003-075, 003-25)

Comments

- Binding capacity of minicolumn: up to 20 µg of DNA / Minimum binding capacity of minicolumn: 2 µg DNA

  If DNA is below 2 µg we recommend using Gel-Out Concentrator Kit (cat.# 023-50C, 023-250C)
- DNA fragments range: 100-10 000 bp
- Typical DNA recovery: 60-80%
- Elution volume: 30-50 µl

Isolation protocol

1. Cut out the agarose slices (up to 200 mg) containing DNA. Transfer agarose slices to Eppendorf tubes (not included).

   Agarose gel electrophoresis can be performed in the presence of either TAE or TBE buffer.

2. Add an appropriate volume of **R7SI** agarose melting solution:
   < 2% agarose gel - **400 µl**
   ≥ 2% agarose gel - **500 µl**

   Incubate samples at **50 °C** until complete dissolution of agarose silices. Mix the samples by inverting the tubes or vortexing a few times.

   Agarose melting solution R7SI contains the color pH indicator. Upon mixing the DNA sample with R7SI agarose melting solution. Yellow color of the mixture indicates an optimal pH for DNA binding.

   If the mixture color turns pink the pH of the solution is too high. In such conditions DNA binds ineffectively to the silica membranes and may be lost.

   Too high pH can be corrected by adding 1-10 µl of 3M sodium acetate solution (pH 5.5) (included) and mix. Purification can be continued after reaching a yellow color.

    optimal condition pH ≤7.2

    too high pH

3. Add an appropriate volume of **isopropanol**:
   < 2% agarose gel - **200 µl**
   ≥ 2% agarose gel - **250 µl**

   Mix by inverting the tubes.

4. Briefly centrifuge the samples to remove the leftovers of solution from the tube walls and caps.

5. Apply samples onto the minicolumns.

6. Centrifuge for **30 s** at **10 000-15 000 RPM**.

7. Remove the minicolumns, discard the filtrate. Place the minicolumns to **the same** tubes.

8. Add **600 µl** of **A1** wash solution.

9. Centrifuge for **30 s** at **10 000-15 000 RPM**.

10. Remove the minicolumns, discard the filtrate. Place the minicolumns to **the same** tubes.

11. Add **300 µl** of **A1** wash solution.

12. Centrifuge for **1 min** at **10 000-15 000 RPM**.

13. Remove the minicolumns, discard the filtrate. Place the minicolumns to **the same** tubes.

14. Centrifuge for **1 min** at **10 000-15 000 RPM**.

15. Transfer the minicolumns to **new** 1.5 ml tubes (not included).

16. Add **50 µl** of **TE** buffer or sterile water (not included) directly onto the minicolumn resin.

    Applying elution liquid (TE buffer or sterile water) onto the minicolumn be sure that liquid is applied directly onto the resin. If some of the liquid stays on the column wall the elution will be less effective.
    Elution in a smaller volume is less efficient, but the extracted DNA has a higher concentration. Elution in 50 µl volume is more efficient, but DNA has a lower concentration.

17. Incubate for **3 min** at **room temp.**

18. Centrifuge for **1 min** at **10 000-15 000 RPM**.

17. Remove the minicolumns and store the tubes with purified DNA at **4-8°C** until later use.

Safety information

WARNING

R7SI agarose melting 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.

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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