

Manual

Gel-Out Concentrator

Kit for DNA extraction from agarose. Low elution volume (from 15 µl)

catalog #	size
023-50C	50 isolations
023-250C	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

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Contents

component	023-50C	023-250C	storage
Microcolumns	50 pcs	250 pcs	room temp.
1.5 ml elution tubes	50 pcs	250 pcs	room temp.
R7SI agarose melting solution	30 ml	140 ml	room temp.
A1 wash solution	30 ml	140 ml	room temp.
Sodium acetate (3M, pH 5.5)	500 µl	3 ml	room temp.
Isopropanol	15 ml	70 ml	room temp.
Tris buffer (10 mM, pH 8.5)	2 ml	9 ml	room temp.

Additional equipment and reagents

Necessary

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

Comments

- Binding capacity of microcolumn: up to 10 µg of DNA
- DNA fragments range: 100-10 000 bp
- Typical DNA recovery: 65-80%
- Elution volume: 15-30 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 microcolumns. Close the tubes with the caps.

6. Centrifuge for **30-60 s** at **10 000-15 000 RPM**.

7. Remove the microcolumns, discard the filtrates. Place the microcolumns to **the same** tubes.

8. Add **300 µl** of **A1** wash solution. Close the tubes with the caps.

9. Centrifuge for **30-60 s** at **10 000-15 000 RPM**.

10. Add **200 µl** of **A1** wash solution. Close the tubes with the caps.

11. Centrifuge for **2 min** at **10 000-15 000 RPM**.

12. Transfer the microcolumns to **new** 1.5 ml tubes (not included).

13. Add **15-30 µl** of **Tris** buffer directly onto the microcolumn resin. Close the tubes with the caps.

Applying Tris buffer onto the column 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 30 µl is recommended for fragments over 2000 bp.

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

15. Centrifuge for **2 min** at **10 000-15 000 RPM**.

16. Remove the microcolumns, close the tubes. Store the tubes with purified DNA at **4-8°C** until later use.

Elution tube has a long elastic cap connector. It's important to start closing the tube by carefully pressing the cap on the connector side. A "click" - sound confirms proper closure. Different ways of closing may cause opening of the tube during storage.

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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