A&A BIOTECHNOLOGY
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Manual

Gel-Out AX

Increased efficiency kit for DNA extraction from low melting point agarose. Procedure with DNA precipitation.

catalog #	size
024-50	50 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	024-50	storage
Spin 10AX columns	50 pcs	2-8 °C
2 ml tubes	50 pcs	15–25 °C
LPD agarose melting solution	30 ml	15–25 °C
K2 wash solution	55 ml	15–25 °C
K4 elution solution	30 ml	15–25 °C
PM precipitation mix	25 ml	15–25 °C
TE buffer	5 ml	15–25 °C

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf tubes
- 70% ethanol
- Incubator or thermoblock set to 60 °C (Eppendorf Thermomixer recommended)
- Vortex
- Microcentrifuge

Additional

- Sterile water (cat.# 003-075, 003-25)
- Tris buffer (10 mM, pH 8.0) (cat.# 202-50, 202-150)

Comments

- Binding capacity of minicolumn: up to 10 µg of DNA
- DNA fragments range: 100-20 000 bp

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 **500 µl** of **LDP** agarose melting solution. Mix the samples.
3.	Incubate at **60 °C** until complete dissolution of agarose silices (5-10 min). Mix the samples by inverting the tubes a few times.
4.	Keep tubes for **3 min** at **room temp**.
5.	Apply samples onto the Spin 10AX columns.
6.	Centrifuge for **30 s** at **5000 RPM**.
7.	Remove the Spin 10AX columns, discard the filtrate. Place the Spin 10AX columns to **the same** tubes.
8.	Add **500 µl** of **K2** wash solution.
9.	Centrifuge for **30 s** at **5000 RPM**.
10.	Remove the Spin 10AX columns, discard the filtrate. Place the Spin 10AX columns to **the same** tubes.
11.	Add **500 µl** of **K2** wash solution.
12.	Centrifuge for **30 s** at **5000 RPM**.
13.	Transfer the Spin 10AX columns to **new** 2 ml tubes (included).
14.	Add **250 µl** of **K4** elution solution. Keep tubes for **2 min** at **room temp.**
15.	Centrifuge for **30 s** at **5000 RPM**.
16.	Add **250 µl** of **K4** elution solution. Keep tubes for **2 min** at **room temp.**

17. Centrifuge for **30 s** at **5000 RPM**. Remove the Spin 10AX columns.

18. PM precipitation mix contains a precipitation enhancer and it should be intensively mixed before use by vigorous hand shaking.

    Add **400 µl** of **PM** precipitation mix.

19. Mix the samples by inverting the tubes a few times. Centrifuge for **10 min** at **12 000 RPM**.

20. Carefully discard supernatants.

    Light-blue DNA pellets should be visible at the bottom of the precipitation tube.

21. Add **500 µl** of **70% ethanol** (not included). Mix the tubes.
    Centrifuge for **3 min** at **12 000 RPM**.

22. Discard supernatants. Air dry DNA pellets for **5 min** at **room temp.** in the up-site-down position.

    If there are any leftovers (small droplets) of alcohol on the tube walls they should be removed with sterile cotton buds.

23. DNA pellets can be dissolved in the desired volume of TE buffer, sterile water (not included) or Tris buffer (not included).

    Blue color of DNA precipitate enables visual confirmation of the DNA dissolution process.

24. Store purified DNA at **4-8°C** until later use.

Safety information

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

K4 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

PM precipitation mix

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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