A&A BIOTECHNOLOGY
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Manual

Plasmid Mini

Kit for high-copy plasmid DNA purification.

catalog #	size
020-50	50 isolations
020-250	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

Specification

form	minicolumn
binding capacity	20 µg of DNA
sample size	up to 3 ml of bacteria culture
elution volume	from 60 µg
elution solution	TE buffer, water

Contents

component	020-50	020-250	storage
Minicolumns	50 pcs	250 pcs	15–25 °C
L1 cell suspension solution	11 ml	60 ml	2–8 °C
L2 lysis solution	11 ml	60 ml	15–25 °C
GL3 neutralizing solution	25 ml	125 ml	15–25 °C
W first wash solution	30 ml	150 ml	15–25 °C
A1 second wash solution	50 ml	200 ml	15–25 °C
TE buffer	5 ml	16 ml	15–25 °C

Additional equipment and reagents

Necessary

- Centrifuge
- 1,5 ml sterile Eppendorf tubes

Optional

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

Important notes

- Kit contains the LySee color system for easy optical control of alkaline lysis progress (page 6).
- SDS detergent is a component of L2 lysis solution and precipitates at low temperatures. Whenever the L2 lysis solution is not clearly transparent it must be warmed at 40 °C to form a thoroughly clear solution.

Protocol

1. Centrifuge up to **3 ml (1,5-3 ml)** of overnight bacterial culture.

2. Discard the supernatant. Suspend the bacterial pellet in **200 µl** of **L1** cell suspension solution.

   Note. During the pellet bacterial suspension, the solution will change color from a transparent deep pink to opaque light pink. The suspension is completed with complete disappearance of the pellet at the bottom tube.

3. Add **200 µl** of **L2** lysis solution and gently mix. Keep for **3 min** at **room temp**.

   Note. After the addition of L2 lysis solution, gently mix the tube so as not to cause fragmentation of the chromosomal DNA. Gently mix the tube by inverting a few times. The mixture should change appearance and color. After 3 min of incubation, the lysate must be completely clear and uniformly raspberry. If not, mix the lysate a few times and incubate again for 3 min at room temp.

4. Add **400 µl** of **GL3** neutralizing solution and gently mix until the disappearance of the raspberry color of the lysate.

   Note. After the addition of L3T neutralizing solution followed by the rapid precipitation of the potassium salts (SDS), chromosomal DNA and certain proteins. After mixing, the tube contents should change the color to yellowish. No traces of raspberry color indicates complete neutralization and successful ending of the alkaline lysis.

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

6. Apply the lysate (supernatant) onto the minicolumn.

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

8. Remove the minicolumns from the tubes. Discard the filtrates. Place the minicolumns to **the same** tubes.

9. Add **500 µl** of **W** first wash solution.

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

11. Remove the minicolumns from the tubes. Discard the filtrates. Place the minicolumns to **the same** tubes.

12. Add **600 µl** of **A1** second wash solution.

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

14. Transfer the minicolumns to a new 1.5 ml tube (not included).

15. Add **60 µl** of **TE** buffer (included) or **sterile wate**r (not included) directly onto the minicolumn resin.

16. Keep the sample for **3 min** at **room temp**.

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

18. Remove the minicolumns. Store the plasmid DNA at 4-8 °C until later use.

LySee color system

The LySee color system enables an easy and convenient visual control of alkaline lysis. The visual control system prevents common handling errors of incomplete cell resuspension, inefficient cell lysis and incomplete precipitation of unwanted cell components.

Resuspension and lysis

The addition of the transparent purple L1 color cell suspension solution to the bacterial cell pellet makes the bacterial cell pellet easy to localize (fig 1). During the suspension of the bacterial cell pellet, the solution turns opaque light pink (fig 2). The suspension is completed with the complete disappearance of the pellet at the bottom of the tube. After the addition of L2 lysis solution and incubation, lisate turns transparent raspberry. Cell lysis is completed when the solution will turn homogeneously transparent raspberry (fig 3).

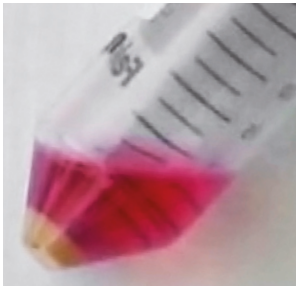

fig 1

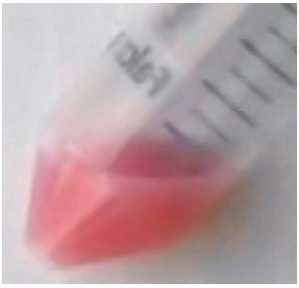

fig 2

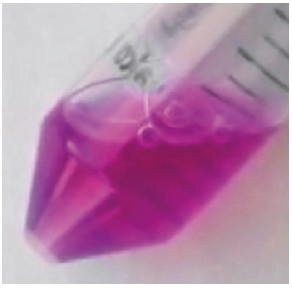

fig 3

Neutralization and precipitation

The addition of the GL3 neutralizing solution causes rapid precipitation of potassium salts (SDS), chromosomal DNA and some proteins (fig 4). After mixing, the solution turns yellowish (fig 5). No traces of raspberry color indicates complete neutralization and successful ending of alkaline lysis (fig 6).

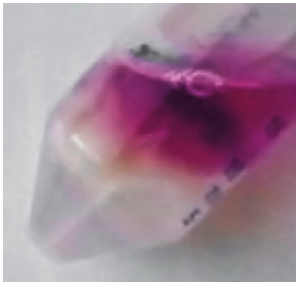

fig 4

fig 5

fig 6

Safety Information

DANGER

A1 second 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.

WARNING

L2 lysis solution

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.

DANGER

GL3 neutralizing solution

H315 Causes skin irritation.
H318 Causes serious eye damage.
H335 May cause respiratory irritation.
P261 Avoid breathing vapors.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
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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