A&A BIOTECHNOLOGY
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Plasmid Mini

Kit for high-copy plasmid DNA purification.
version 0617

50 isolations, 250 isolations

Cat. # 020-50, 020-250

The binding capacity of the plasmid purification minicolumn is 20 µg of DNA.

For R&D use only.

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Kit Contents

Component	50 isolations	250 isolations	Store at
Minicolumns	25 pcs	250 pcs	Room Temp.
L1 colour cell suspension solution	12.5 ml	60 ml	Room Temp.
L2 lysis solution	12.5 ml	60 ml	Room Temp.
GL3 neutralizing solution	25 ml	125 ml	Room Temp.
W first wash solution	30 ml	150 ml	Room Temp.
A1 second wash solution	50 ml	200 ml	Room Temp.
TE buffer	5 ml	16 ml	Room Temp.

Equipment and materials necessary for DNA isolation that are not included in kit

1. Material for plasmid DNA isolation
2. Centrifuge
3. Sterile water (nuclease free, DEPC treated) (cat. # 003-075, 003-25) (option)
4. 1.5 ml sterile Eppendorf tubes

NOTE:
Before you start working, we recommend cleaning the work surface using LabZAP™ product (cat. # 040-500)

A&A Biotechnology provides one year guarantee on this kit
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- not recommended use of equipment and materials
- the use of other reagents than recommended or which are not a component of the kit
- the use of expired or improperly stored reagents and columns

Protocol

Note:
1. Plasmid Mini kit contains the LySee colour system for easy optical control of alkaline lysis progress (more information – page 5).
2. SDS detergent is a component of L2 lysis solution and precipitates at lower temperatures. Whenever the L2 lysis solution is not clearly transparent it must be warmed at 40 ºC to form a thoroughly clear solution.

1. Centrifuge up to 3 ml (1,5–3 ml) of overnight bacterial culture.
   Remove the supernatants. Suspend the bacterial pellets in 200 µl of L1 cell suspension solution.

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

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

   After addition of L2 lysis solution, gently mix the tube contents so as not to cause fragmentation of the chromosomal DNA. Gently mix the tube by inverting (5–6 times). The mixture should change appearance and colour.

   After 3 min of incubation, the lysate must be completely clear and uniformly raspberry. If not, mix the lysate several times and prolong the incubation time for a further 3 min.

3. Add 400 µl of GL3 neutralizing solution and gently mix until the disappearance of raspberry colour of the lysates.

   After addition of GL3 neutralizing solution followed by rapid precipitation of the potassium salts (SDS), chromosomal DNA and certain proteins. After mixing, the tube contents should change the colour to yellowish. No traces of raspberry colour indicates complete neutralization and successful ending of the alkaline lysis.

4. Centrifuge the lisates for 10 min at 10 000–15 000 RPM.

5. Transfer the supernatants onto the minicolumns.

6. Centrifuge for 1 min at 10 000–15 000 RPM.

7. Remove the minicolumns from the tubes, discard the filtrates and re-attach the minicolumns to the same tubes.

8. Add 500 µl of W first wash solution.

9. Centrifuge for 1 min at 10 000–15 000 RPM.

10. Remove the minicolumns from the tubes, discard the filtrates and re-attach the minicolumns to the same tubes.

11. Add 600 µl of A1 second wash solution.

12. Centrifuge for 2 min at 10 000–15 000 RPM.

13. Transfer the dry minicolumns to new 1.5 ml tubes.

    Add 60 µl of TE buffer (included) or sterile water (not included) directly onto the minicolumns resin.

14 Incubate for 3 min at room temp.

15. Centrifuge for 1 min at 10 000–12 000 RPM.

16. Remove the minicolumns.
    Store the plasmid DNA at +4 °C to +8 °C.

LySee System

LySee 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.

Direct visual control of a cell resuspension, lysis, neutralization and precipitation:

1. Resuspension and lysis:

The addition of the transparent purple L1 colour 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 homogenously transparent raspberry (fig. 3).

fig. 1

fig. 2

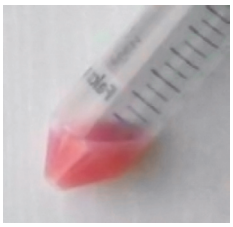

fig. 3

2. 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 colour indicates complete neutralization and successful ending of alkaline lysis (fig. 6).

fig. 4

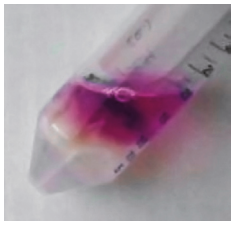

fig. 5

fig. 6

Related products

Product	Quantity	Cat. #
Plasmid Mini AX	50 isolations	010-50
Plasmid Mini AX Gravity	100 isolations	015-100
Plasmid Midi AX	10 isolations	092-10
Plasmid Maxi AX Sil	2 isolations	093-02S
E.coli Transformer Kit	6x40 reactions	4020-240
Saccharomyces Transformer Kit	120 reactions	4010-120
Pichia Transformer Kit	120 reactions	4000-120
KineX	20 reactions	1008-20
	100 reactions	1008-100
OverLap™ Assembly	10 reactions	1024-10
	50 reactions	1024-50
T4 DNA Ligase	200 U	1004-200
	1000 U	1004-1000

Safety information

DANGER

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 vapours.
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.

DANGER

A1 second wash solution

H225 Highly flammable liquid and vapour.
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 vapours.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Notes: