A&A BIOTECHNOLOGY
innovating life science

Plasmid Midi AX

Increased efficiency kit for low- and high-copy plasmid DNA purification. Procedure with DNA precipitation.

version 0617

10 isolations

Cat. # 092-10

The binding capacity of the plasmid purification column is 200 µg of DNA.

For R&D use only.

A&A BIOTECHNOLOGY, Aleja Zwycięstwa 96/98, 81-451 Gdynia, Poland
tel: +48 58 6982194, +48 883 323 761, **fax:** +48 58 6228578
VAT PL584-025-44-03
info@aabiot.com www.aabiot.com

Kit Contents

Component	10 isolations	Store at
Plasmid 200 columns	10 pcs	+4 to +8 °C
50 ml tubes	10 pcs	Room Temp.
Filtration tubes	10 pcs	Room Temp.
Counterweight column	1 pcs	Room Temp.
L1 colour cell suspension solution	55 ml	Room Temp.
L2 lysis solution	55 ml	Room Temp.
L3 neutralizing solution	55 ml	Room Temp.
K2P wash solution	220 ml	Room Temp.
K3 elution solution	90 ml	Room Temp.
Precipitation enhancer	350 µl	Room Temp.
TE buffer	16 ml	Room Temp.
Isopropanol	60 ml	Room Temp.

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

1. Material for plasmid DNA isolation
2. 70% ethanol
3. Centrifuge
4. Sterile water (nuclease free, DEPC treated) (cat. # 003-075, 003-25) (option)
5. 15 ml / 50 ml sterile Falcon 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 Midi AX kit contains the LySee colour system for easy optical control of alkaline lysis progress (more information – page 6).
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 100 ml (50–100 ml) of a overnight bacterial culture. Remove the supernatants. Suspend the bacterial pellets in 5 ml 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 5 ml of L2 lysis solution and gently mix by inverting the tubes. Incubate for 5 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 5 ml of L3 neutralizing solution and gently mix until the disappearance of raspberry colour of the lysates.

   After addition of L3 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. Apply the lysates onto the filtration tubes, close the filtration tubes and centrifuge for 5 min at 1500 x g.

5. During the centrifugation prepare the Plasmid 200 columns. Place them into 50 ml tubes (included) and set the columns with tubes in the suitable rack.

6. Remove the fliters and apply the clear lysates (from point 4) onto Plasmid 200 columns (from point 5).

   Wait until the lysates pass through the Plasmid 200 columns.

7. Add 20 ml of K2P wash solution.

   Wait until the K2P wash solution passes through the Plasmid 200 columns.

8. Transfer the Plasmid 200 columns to new 50 ml Falcon tubes (not included).

   Add 6 ml of K3 elution solution.

   Wait until the K3 elution solution passes through the Plasmid 200 columns.

9. Transfer the eluates to new 15 ml Falcon tubes (not included).

   Add 25 µl of precipitation enhancer and 5 ml of Isopropanol.

   In situation when it is not necessary or is not desirable to add the precipitation enhancer, add only 5 ml of isopropanol. The efficiency of the isolation will not be reduced.

10. Mix the samples by inverting the tubes a few times and centrifuge for 10 min at 11 000 x g.

11. Carefully discard the supernatants.

    Add 2 ml of 70% ethanol (not included).
    Mix the samples and centrifuge for 3 min at 11 000 x g.

    The light-blue DNA pellet should be visible at the bottom of the precipitation tube.

12. Carefully discard the supernatants and air dry the DNA pellets for 10 min at room temp. up-side-down.

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

13. Dried DNA pellets can be dissolved in 0.2–1 ml of TE buffer (included) or sterile water (not included).

    The blue color of DNA precipitate enables visual confirmation of the DNA dissolution process.

14. Store the plasmid DNA at +4 ºC to +8 ºC.

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

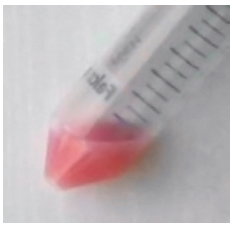

fig. 3

2. Neutralization and precipitation:

The addition of the L3 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

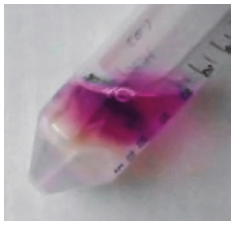

fig. 5

fig. 6

Related products

Product	Quantity	Cat. #
Plasmid Mini	50 isolations	020-50
	250 isolations	020-250
Plasmid Mini AX Gravity	100 isolations	015-100
Plasmid Mini AX	50 isolations	010-50
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

K3 elution 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.

DANGER

Isopropanol

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.