A&A BIOTECHNOLOGY
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Manual

Plasmid Midi AX Endotoxin-Free

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

catalog #	size
092EF-10	10 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	midicolumn
binding capacity	200 µg of DNA
sample size	up to 100 ml of bacteria culture
elution volume	precipitation

Contents

component	size	cat #	storage
Plasmid 200 columns	10 pcs	K-P200-10	2–8 °C
Tubes 50 ml	2 x 5 pcs	K-F50-5	15–25 °C
Filtration columns	2 x 5 pcs	K-WF50-5	15–25 °C
Counterweight column	1 pc	K-CW50-1	15–25 °C
LPS-out endotoxin removal solution	15 ml	K-LPS-15	15–25 °C
L1 cell suspension solution	60 ml	K-L1-60	2–8 °C
L2 lysis solution	60 ml	K-L2-60	15–25 °C
L3 neutralizing solution	60 ml	K-L3-60	15–25 °C
K2P wash solution	220 ml	K-K2P-220	15–25 °C
K3 elution solution	90 ml	K-K3-90	15–25 °C
TE buffer	15 ml	K-TE-15	15–25 °C
Precipitation enhancer	350 µl	K-WZM-035	15–25 °C
Isopropanol	60 ml	K-IZO-60	15–25 °C

Additional equipment and reagents

Necessary

- Ethanol 70%
- Centrifuge
- 15 ml and 50 ml sterile Falcon 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 **100 ml** of overnight bacterial culture. Discard the supernatant. Suspend the bacterial pellet in **5 ml** 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.

2. Add **5 ml** of **L2** lysis solution and gently mix by inverting the tube. Keep for **5 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.

3. Add **5 ml** of **L3** neutralizing solution and gently mix until the disappearance of the raspberry color of the lysate.

   Note. After the addition of L3 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.

4. Transfer the lysate to the filtration column. Close the tube and centrifuge for **5 min** at **1500 x g**.

5. Add **1.2 ml LPS-out** solution to the filtered lysate. Mix and keep on **ice** for **30 min**.

6. Place the Plasmid 200 column to a **50 ml tube**.

7. Transfer the lysate from point 5. to the Plasmid 200 column. Wait for the lysate to flow through the column.

8. Add **20 ml** of **K2P** wash solution. Wait for the solution to flow through the column.

9. Transfer the Plasmid 200 column to a **new** 50 ml tube (not included).

10. Add **6 ml** of **K3** elution solution. Wait for the eluate to flow through the column.

11. Transfer the eluate to a **new** 15 ml tube (not included).

12. Add **25 µl** of **precipitation enhancer** and **5 ml** of **isopropanol**.

Note. In situations where it is not necessary or not desirable to add a precipitation enhancer (e.g. very sensitive transfection), only 5 ml of isopropanol should be added. This will not reduce the isolation efficiency.

13. Mix the sample by inverting the tube a few times and centrifuge for **10 min** at **11 000 x g**.

14. Carefully discard supernatant. Be careful not to remove the DNA pellet at the bottom of the tube.

Attention. When pouring out the supernatant, it is very easy to lose the DNA pellet. For safety, it is recommended to pour the supernatant into the prepared tube so the pellet can be recovered.

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

16. Carefully discard supernatant. Be careful not to remove the DNA pellet at the bottom of the tube.

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

17. Air dry the plasmid DNA pellet for **10 min** at room temp. up-site down.

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

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

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

19. Store the plasmid DNA at 4-8 °C.

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).

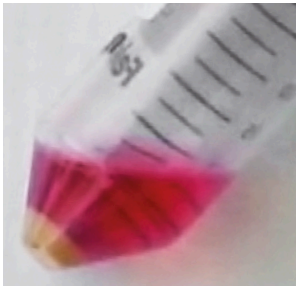

fig 1

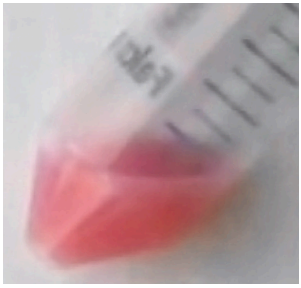

fig 2

fig 3

Neutralization and precipitation

The addition of the 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 color indicates complete neutralization and successful ending of alkaline lysis (fig 6).

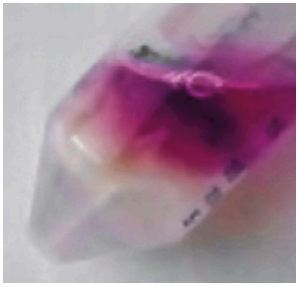

fig 4

fig 5

fig 6

Safety Information

LPS-out endotoxin removal solution

DANGER

H225 Highly flammable liquid and vapor.
H315 Causes skin irritation
H319 Causes serious eye irritation.
H335 May cause respiratory irritation
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
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.

L2 lysis solution

WARNING

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.

K3 elution solution

DANGER

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.

Isopropanol

DANGER

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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