A&A BIOTECHNOLOGY
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Manual

Plasmid Maxi AX Sil Endotoxin-Free

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

catalog #	size
093EF-02S	2 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	maxicolumn
binding capacity	1 mg of DNA
sample size	up to 500 ml of bacteria culture
elution volume	precipitation

Contents

component	size	cat #	storage
Maxi AX Sil 1000 columns	2 pcs	K-P1000-2	15–25 °C
Tubes 50 ml	2 x 5 pcs	K-F50-5	15–25 °C
Filtration syringes	2 pcs	K-SF60-2	15–25 °C
LPS-out endotoxin removal solution	10 ml	K-LPS-10	15–25 °C
L1 cell suspension solution	45 ml	K-L1-45	2–8 °C
L2 lysis solution	45 ml	K-L2-45	15–25 °C
L3 neutralizing solution	45 ml	K-L3-45	15–25 °C
K1 Sil equilibrating solution	55 ml	K-K1S-55	15–25 °C
K2 Sil wash solution	130 ml	K-K2S-130	15–25 °C
K3 elution solution	55 ml	K-K3-55	15–25 °C
TE buffer	5 ml	K-TE-5	15–25 °C
Isopropanol	50 ml	K-IZO-50	15–25 °C

Additional equipment and reagents

Necessary

- Ethanol 70%
- Centrifuge
- Laboratory beakers

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 **200** to **500 ml** of overnight bacterial culture.

   Note. Volume of bacterial culture that can be processed depends on the particular plasmid copy number. Please keep in mind that the total binding capacity of the column is 1 mg DNA.

2. Discard supernatants. Suspend the bacterial pellet in **20 ml** of **L1** cell suspension solution.

   Note. During the bacterial pellet suspension, the solution will change color from a transparent deep pink to opaque light pink. Suspending is considered completed when the pellet, at the bottom of the tube disappears.

3. Prepare two **tubes 50 ml** (included). Transfer **10 ml** of the suspension to each of the two tubes.

4. Add **10 ml** of **L2** lysis solution to each tube. Gently mix by inverting the tubes.

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

5. Keep for **3 min** at **room temp**.

   Note. After 3 min, the lysate should be clear and raspberry in color. If not, mix the lysate several times and leave for an additional 3 min.

6. Add **10 ml** of **L3** neutralizing solution to each tube. Gently mix until the raspberry color disappears.

   Uwaga. After the addition of L3 neutralizing buffer 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.

7. Keep on ice for **15 min**.

8. Centrifuge for **5 min** at **10 000 x g**.

9. Remove the plunger from the filtration syringe and pour the contents of both 50 ml tubes into the filtration syringe.

10. Prepare two **new** tubes 50 ml (included). Insert the plunger into the filtration syringe and filter **30 ml** of the lysate into each of the two tubes.

11. Add **2.4 ml** of **LPS-out** solution to each of the two tubes.

12. Close tubes, mix and keep on ice for **30 min**.

13. Prepare the Maxi AX Sil 1000 column by placing it in the upper opening of the rack. Place a beaker under the column to collect waste.

14. Add **25 ml** of **K1 Sil** equilibrating solution to the Maxi AX Sil 1000 column. Wait for the solution to flow through the column.

15. Pour the content of both **50 ml tubes** from point 12. of the protocol into the column. Wait for the lysate to flow through the column.

16. Add **30 ml** of **K2 Sil** wash solution to the column. Wait for the solution to flow through the column.

17. Again, add **30 ml** of **K2 Sil** wash solution to the column. Wait for the solution to flow through the column.

18. Remove the beaker under the column. Place a **tube 50 ml** (included) in the bottom hole of the rack, directly below the column.

19. Add **25 ml** of **K3** elution solution to the column. Wait for the eluate to flow through the column.

20. Remove the Maxi AX Sil 1000 column from the rack and add **20 ml** of **isopropanol** to the tube.

21. Close the tube, mix and centrifuge for **20 min** at **11 000 x g**.

22. 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. To be safe, it is recommended to pour the supernatant into the prepared tube so the pellet can be recovered.

23. Add **2 ml** of **70% ethanol** to the tube. Gently mix and centrifuge for **3 min** at **11 000 x g**.

24. 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. To be safe, it is recommended to pour the supernatant into the prepared tube so the pellet can be recovered.

25. 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.

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

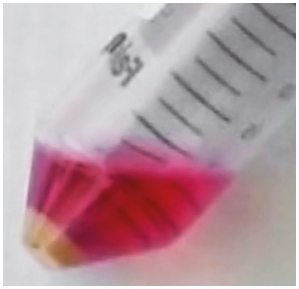

fig 1

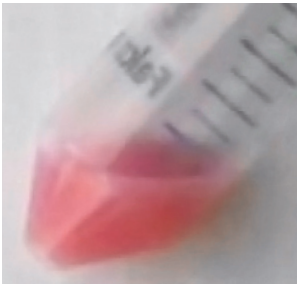

fig 2

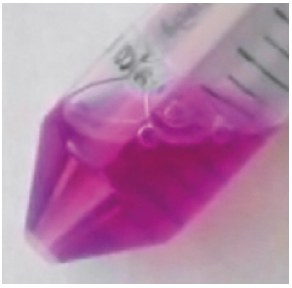

fig 3

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).

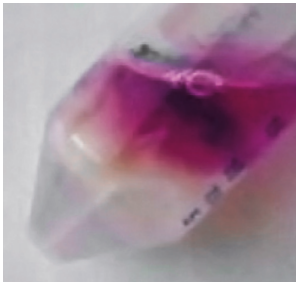

fig 4

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.

K1 Sil equilibrating 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.

K2 Sil wash 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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