User Bulletin

VIOGENE
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VER.:17A

Low-Endotoxin Plasmid DNA Miniprep Extraction System

Viogene Low-Endotoxin Plasmid DNA Miniprep Extraction System provides a simple, fast and cost-effective method to purify plasmid DNA without phenol/chloroform extraction. It is based on binding of DNA to silica-based membranes in chaotropic salts. An average yield of 1 to 40 μg of plasmid DNA can be expected from 1 to 5 ml overnight bacterial culture.

Downstream Application

- Restriction digestion
- Radioactive and fluorescent sequencing
- Transformation / Transfection
- Ligation
- PCR, RAPD
- NGS
- Gene editing

Product Contents

Cat. No	GFN1001	GFN1002
Preps	50	250
MX1 Buffer	12ml	60ml
MX2 Buffer	15ml	75ml
MX3 Buffer	20ml	100ml
WE Buffer	30ml	150ml
WS Buffer	10ml	45ml
RNase A (20mg/ml)	0.042ml	0.210ml
Mini *Plus* Column	50	250
Collection Tube	50	250
Protocol	1	1

All buffers need to be mixed well before use.

Shipping & Storage

Viogene Low-Endotoxin Plasmid DNA Miniprep Extraction System should be shipped and stored at ambient temperature for up to 12 months.

If precipitate form by freezing temperature on any buffer, warm up at 37℃ to redissolve.

Protocol

- **Please read the following notes before starting the procedures.**

Important Notes

- Add all of RNase A (20mg/ml, 0.042 or 0.210ml) into the MX1 Buffer and mix well, store at 4℃.
- Buffers provided in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn.
- If precipitation forms in MX2 Buffer, incubate the buffer at 55 ℃ for 10 minutes to redissolve the salt precipitates.
- Do not shake MX2 Buffer, SDS in MX2 will lead to serious foaming.
- All procedures should be done at room temperature (20 - 25 ℃).
- All centrifugation steps are done at 7,000 x g - 10,000 x g (9,000 rpm - 13,000 rpm) in a microcentrifuge, if not notice.
- For long-term storage of the eluted plasmid, TE buffer should be used for elution. Since EDTA in TE may affect downstream applications, endotoxin-free ddH_2O (pH 7.0 - 8.5) is preferred for DNA elution immediately used for further enzymatic reactions.

For GF2001

- Add 40 ml of 98 - 100 % ethanol into WS Buffer bottle when first open.

For GF2002

- Add 180 ml of 98 - 100 % ethanol into WS Buffer bottle when first open.

Viogene's unique design — EasyLid

The EasyLid is designed to prevent contamination during the procedure.

Twist the arm of the cap and pull the cap to break the EasyLid.

I. Protocol for Spin Method:

1. Grow 1 to 5 ml plasmid-containing bacterial cells in LB medium with appropriate antibiotic(s) overnight (12-16 hours) with vigorous agitation.

2. Pellet the cells by centrifuging for 1 - 2 minutes. Decant the supernatant and remove all medium residue by pipetting.

3. Add 200 μl of MX1 Buffer to the pellet, resuspend the cells completely by vortexing or pipetting.

No cell clumps should be visible after resuspension of the pellet.

4. Add 250 μl of MX2 Buffer and gently mix (invert the tube 4-6 times) to lyse the cells until the lysate becomes clear. Incubate at room temperature for 1 - 5 minutes.

Do not vortex, vortexing will shear genomic DNA.

5. Add 350 μl of MX3 Buffer to neutralize the lysate, then immediately and gently mix the solution.

The white precipitate should be formed.

6. Centrifuge at 10,000 x g (13,000 rpm) for 5 - 10 minutes, meanwhile place a Mini *Plus* Column onto a Collection Tube.

7. Transfer the supernatant carefully into the column.

8. Centrifuge at 7,000 x g (9,000 rpm) for 30 - 60 seconds. Discard the flow-through.

9. Wash the column once with 0.5 ml WE Buffer by centrifuging at 7,000 x g (9,000 rpm) for 30 - 60 seconds. Discard the flow-through.

10. Wash the column once with 0.7 ml WS Buffer by centrifuging at 7,000 x g (9,000 rpm) for 30 - 60 seconds. Discard the flow-through.

11. Centrifuge the column at 10,000 x g (13,000 rpm) for another 3 minutes to remove residual ethanol.

It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.

12. Place the column onto a new 1.5-ml centrifuge tube. Add 50 μl of endotoxin-free ddH_2O onto the center of the membrane.

For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.

13. Stand the column for 2 - 3 minutes and centrifuge at 10,000 x g (13,000 rpm) for 2 - 3 minutes to elute DNA.

14. Store plasmid DNA at 4 ℃ or –20 ℃.

II. Protocol for Vacuum Method:

1. Grow 1 to 5 ml plasmid-containing bacterial cells in LB medium with appropriate antibiotic(s) overnight (12-16 hours) with vigorous agitation.

2. Pellet the cells by centrifuging for 1 - 2 minutes. Decant the supernatant and remove all medium residue by pipetting.

3. Add 200 μl of MX1 Buffer to the pellet, resuspend the cells completely by vortexing or pipetting.
No cell clumps should be visible after resuspension of the pellet.

4. Add 250 μl of MX2 Buffer and gently mix (invert the tube 4-6 times) to lyse the cells until the lysate becomes clear. Incubate at room temperature for 1 - 5 minutes.
Do not vortex, vortexing will shear genomic DNA.

5. Add 350 μl of MX3 Buffer to neutralize the lysate, then immediately and gently mix the solution.
The white precipitate should be formed.

6. Centrifuge at 10,000 x g (13,000 rpm) for 5 - 10 minutes, meanwhile insert the tip of a Mini *Plus* Column into the luer-lock of a vacuum manifold (e.g. Promega's Vac-man*).

7. Transfer the supernatant carefully into the column.

8. Apply vacuum to draw all the liquid into the manifold.

9. Wash the column once with 0.5 ml WE Buffer by re-applying vacuum to draw all the liquid.

10. Wash the column once with 0.7 ml WS Buffer by re-applying vacuum to draw all the liquid.
It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.

11. Place the column onto a Collection Tube. Centrifuge the column at 10,000 x g (13,000 rpm) for another 3 minutes to remove residual ethanol.
It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.

12. Place the column onto a new 1.5 ml centrifuge tube. Add 50 μl of endotoxin-free ddH_2O onto the center of the membrane.
For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.

13. Stand the column for 2 - 3 minutes and centrifuge at 10,000 x g (13,000 rpm) for 2 - 3 minutes to elute DNA.

14. Store plasmid DNA at 4 ℃ or –20 ℃.

- Vac-man is a trademark of Promega Corporation.