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VIOGENE

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User Bulletin

Low-Endotoxin Plasmid DNA Maxiprep Extraction System

Viogene Low-Endotoxin Plasmid DNA Maxiprep 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 500 μg of plasmid DNA can be expected from 100 ml overnight bacterial culture.

Downstream Application

* Restriction digestion
* Radioactive and fluorescent sequencing
* Transformation / Transfection
* Ligation
* PCR, RAPD

Product Contents

Cat. No	GFM1001	GFM1002
Preps	10	25
MX1 Buffer	45ml	105ml
MX2 Buffer	55ml	130ml
MX3 Buffer	75ml	180ml
WE Buffer	225ml	265ml x 2
WS Buffer	35ml	52ml x 3
RNase A (20mg/ml)	0.16ml	0.37ml
Maxi *Plus*™ Column	10	25
Protocol	1	1

All buffers need to be mixed well before use.

Shipping & Storage

Viogene Low-Endotoxin Plasmid DNA Maxiprep 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.

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

Important Notes

- Add all of RNase A (20mg/ml, 0.16 or 0.37ml) 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 ℃).
- 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.

For GFM1001

Add 140 ml of 98 - 100 % ethanol into WS Buffer bottle when first open.

For GFM1002

Add 208 ml of 98 - 100 % ethanol into WS Buffer bottle when first open.

Protocol for Centrifugation Method:

1. **Grow 100 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 10 minutes. Decant the supernatant and remove all medium residue by pipetting.**

3. **Add 4ml of MX1 Buffer (RNase A added) to the pellet, resuspend the cells completely by vortexing or pipetting.**
   No cell clumps should be visible after resuspension of the pellet.

4. **Add 5ml 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 7ml of MX3 Buffer to neutralize the lysate, then immediately and gently mix the solution.**
   The white precipitate should be formed.

6. **Centrifuge cell lysate at 10,000 x g (13,000 rpm) for 5 - 10 minutes, meanwhile place a Maxi *Plus*™ Column onto a 50ml Falcon tube.**

7. **Transfer the supernatant carefully into the Maxi *Plus*™ column.**

8. **Centrifuge at 3,000 x g (3,500 - 4,000 rpm) for 5 - 10 minutes in a swing-bucket centrifuge (in balance). Discard the flow-through.**

9. **Wash the column two times with 10 ml WE Buffer by centrifuging at 3,000 x g (3.500 - 4,000 rpm) for 5 – 10 minutes each time. Discard the flow-through.**

10. **Wash the column two times with 15 ml WS Buffer (ethanol added) by centrifuging at 3,000 x g (3.500 - 4,000 rpm) for 5 – 10 minutes each time. Discard the flow-through.**

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

12. **Centrifuging at 3,000 x g (3.500 - 4,000 rpm) for 5 minutes to elute DNA, collect the eluted DNA (about 1.3 ml) to a new 1.5 ml tube, there will be about 200 μl void volume on the membrane.**

13. **Add another 1.5ml of endotoxin-free ddH_2O onto the center of the membrane and centrifuging at 3,000 x g (3.500 - 4,000 rpm) for 5 minutes to elute DNA the second time, collect the eluted DNA to a new tube.**

14. **Add another 1.5ml of endotoxin-free ddH_2O onto the center of the membrane and centrifuging at 3,000 x g (3.500 - 4,000 rpm) for 5 minutes to elute DNA the third time, collect the eluted DNA to a new tube.**

15. **Each time the amount of eluted DNA will be different, usually the second time elution has the highest amount, one can pool all three elution, if DNA is too diluted, ethanol precipitation or SpeedVac concentration can be applied to obtain desire concentration, and store plasmid DNA at 4 ℃ or –20 ℃.**