Viogene-BioTek Corporation
10F, No.276, Da-Tung Rd.,Sec. 1. Shijr,
New Taipei City, Taiwan,ROC
Tel: +886-2-2647 2259 Fax: +886-2-2647 5094
www.viogene.com

VIOGENE

User Bulletin

96 Wells Mini *Plus* Plasmid Extraction System

Viogene 96 Wells Mini Plus Plasmid DNA 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.

Downstream Application

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

Product Contents

Cat. No	GF961001
Preps	96 x 4
MX1 Buffer	100 ml
MX2 Buffer	100 ml
MXB3 Buffer	100 ml
WN Buffer	45 ml
WS Buffer	45 ml
Elution Buffer	50 ml
RNase A (20mg/ml)	0.350ml
GF96 Column plate	4
GF96 Collection plate	4
GF96 Elution plate	4
Protocol	1

All buffers need to be mixed well before use.

Shipping & Storage

The sample of 96 Wells Mini Plus Plasmid DNA Extraction System is shipped and stored at ambient temperature 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, 0.350ml) into the MX1 Buffer and mix well, store at 4℃
- Add 180 ml/bt of 98 - 100 % ethanol into WN Buffer bottle when first open.
- Add 180 ml/bt of 98 - 100 % ethanol into WS Buffer bottle when first open.
- 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, Elution Buffer (provided) or ddH_2O (pH 7.0 - 8.5) is preferred for DNA elution immediately used for further enzymatic reactions.

Protocol for Spin Method:

1. **Grow 1 to 1.3 ml plasmid-containing bacterial cells in LB medium with appropriate antibiotic(s) overnight (12-16 hours) with vigorous agitation.**

2. **Pellet the 96 deep well culture plate containing the overnight culture by centrifuging at 1800 x *g* for 10 minutes.**

3. **After centrifugation, pour off the supernatant and blot the deep well culture plate upside down on an absorbent pad.**

4. **Add 200 μl of Buffer MX1 to the pellet, resuspend the cell pellet completely by pipetting up and down.**
   No cell clumps should be visible after resuspension of the pellet.

5. **Add 200 μl of Buffer MX2 and gently mix by pipetting up and down 3-5 times or seal the plate with parafilm then invert the plate 5-10 times to lyse the cells until the lysate becomes clear. Incubate at room temperature for 3-5 minutes.**
   Do not vortex, vortexing will shear genomic DNA.

6. **Add 200 μl of Buffer MXB3 to neutralize the lysate and gently mix by pipetting up and down 3-5 times or seal the plate with parafilm then invert the plate 5-10 times.** The white precipitate should be formed.

7. **Centrifuge the deep well culture plate at 3,500-4,500 g for 20 minutes, meanwhile place a GF96 Column plate onto a GF96 Collection plate.**

8. **Transfer the supernatant carefully into the GF96 Column plate.**

9. **Centrifuge at 3,500-4,500 g for 5 minutes. Discard the flow-through.**
   IF there are residual lysates on the column well, centrifuge at 3,500-4,500 g for another 5 minutes.

10. **Wash the well with 0.5 ml WN Buffer by centrifuging at 3,500-4,500 g for 3-5 minutes. Discard the flow-through.**

11. **Wash the well with 0.5 ml WS Buffer by centrifuging at 3,500-4,500 g for 3-5 minutes. Discard the flow-through.**

12. **Centrifuge the GF96 Column Plate at 3,500-4,500 g for another 10 minutes to remove residual ethanol.**
    It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.

13. **Place the GF96 Column Plate onto a GF96 Elution Plate. Add 50-100 μl of Elution Buffer (provided) 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.

14. **Stand the GF96 Column Plate for 5 minutes and centrifuge at 3,500-4,500 g for another 10 minutes to elute DNA.**

15. **Store plasmid DNA at 4 ℃ or –20 ℃.**