



DATA SHEET

Endotoxin-Free Ultrapure Plasmid Extraction Maxiprep System

Viogene Endotoxin-Free Ultrapure Plasmid Extraction Maxiprep System allows the isolation of ultrapure and endotoxin-free plasmid DNA from up to 250 ml culture.

Downstream Application

- * Transfection
- * Transformation
- * Ligation and cloning
- * Sequencing
- * In vitro transcription

Product Contents

Cat. No	GMN1001
Preps	15
VP1 Buffer	200ml
	200ml
VP2 Buffer	200ml
VP3 Buffer	265ml X 4
VPN Buffer	200ml
VPE Buffer	12.5ml
E ² Reagent	1.000ml
RNase A (20mg/ml)	30
	15
Mini Plus Column	1
Maxi Ultraflow Column	
Protocol	
All buffers need to be mixed well before use.	

Shipping & Storage

Viogene Endotoxin-Free Ultrapure Plasmid Extraction Maxiprep System is shipping and storage at ambient temperature up to 12 months.

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

Protocol

- Please read the following notes before starting the procedures.

Important Notes

- Spin RNase A solution tube before use, apply all of RNase A solution into VP1 Buffer bottle and mix well to store at 4°C
- If precipitation forms in VP2 Buffer, incubate at 37°C for 10 minutes to redissolve the salt precipitate. Do not shake VP2 Buffer, SDS present will lead to serious foaming.
- Sit VP3 Buffer on ice before use.
- The volume of VP1-3 Buffer used in the protocol is developed for 100ml sample culture. If the starting sample culture is larger than 100ml, please increase the volume of VP1-3 buffer proportionally.
- Use endotoxin-free equipments, plasticware and glassware for all steps to prevent endotoxin contamination. The use of a laminar flow hood is strongly recommended.
- All reagents & solutions not included in the kit (e.g. isopropanol, 70% ethanol, and TE buffer) should be endotoxin-free grade and freshly prepared with endotoxin-free water.
- Add 50ml Isopropanol into E² Reagent bottle and mix completely by inverting several times, when first open.

<p>1. Culture plasmid-containing bacterial cell in 100-250 ml (high-copy-number plasmids) or 350-500 ml (low-copy-number plasmids) of LB medium. Grow 12-16 hours with vigorous shaking at 37 °C</p> <p>2. Harvest the bacterial cells by centrifugation at 6,000 x g for 15 minutes.</p> <p>3. Equilibrate Maxi Ultraflow™ Columns by applying 5 ml of 98% ethanol. Allow the column to empty by gravity flow and discard the filtrate.</p> <p>4. Apply 10 ml of VPN Buffer to the Maxi Ultraflow™ Column and allow it to flow</p>	<p>The volume of added E² Reagent should be more than 1/10 volume of the supernatant from Step. 8.</p> <p>10. Apply the mixture to the Maxi Ultraflow™ Column and allow it to flow through by gravity flow and discard the filtrate.</p> <p>11. Wash the column twice with 30 ml of VPN Buffer by gravity flow and discard the filtrate.</p> <p>12. Apply 10 ml of VPE Buffer to elute DNA by gravity flow. (Using an endotoxin-free tube to elute DNA can prevent endotoxin contamination in subsequent steps.)</p>
---	---

through by gravity flow and discard the filtrate.

5. Resuspend the cell pellet in 10 ml of VP1 Buffer. The bacterial cells should be completely resuspended before adding VP2 Buffer.

6. Add 10 ml of VP2 Buffer, mix gently by rotating the lysate and stand for 5 minutes. Do not vortex, vortexing will shear genomic DNA. The lysate should be clear and viscous.

7. Add 10 ml of ice-cold VP3 Buffer, mix gently by rotating. After adding VP3 Buffer, white precipitate should be formed.

8. Centrifuge at 20,000 x g for 15 minutes at 4 °C. 20,000 x g corresponds to 12,000 and 13,000 rpm in Beckman JA-17 and Sorvall SS-34 rotors, respectively.

9. Transfer the supernatant into a 50 ml conical tube and add 4 ml of E² Reagent (Isopropanol added). Invert the tube 8~12 times and stand for 2 minutes.

13. Precipitate DNA by adding 7.5 ml (0.75 volumes) of room temperature isopropanol to the elute. Mix and centrifuge at 15,000 x g for 30 minutes at 4°C. Carefully remove the supernatant.

14. Wash the DNA pellet twice with 5 ml of endotoxin-free, room temperature 70 % ethanol and centrifuge at 15,000 x g for 10 minutes. Carefully remove the supernatant.

15. Air-dry the DNA pellet for 10 minutes and dissolve the DNA in 250 µl or a suitable volume of endotoxin-free TE or ddH₂O.

16. To eliminate the insoluble material, load the dissolved DNA sample into a Mini Plus™ Column (sitting in a 1.5 ml tube) and spin at full speed in a microcentrifuge for 20 seconds, collect the eluted DNA sample in the 1.5 ml tube.

17. Store DNA at -20°C.