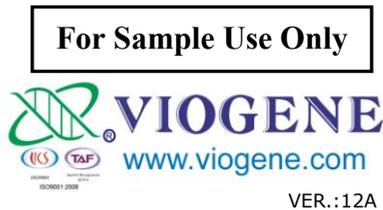


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User Bulletin

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
VP2 Buffer	200ml
VP3 Buffer	200ml
VPN Buffer	265ml X 4
VPE Buffer	200ml
E ² Reagent	12.5ml
RNase A (20mg/ml)	1.000ml
Mini Plus Column	30
Maxi Ultraflow Column	15
Protocol	1

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.

- 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.**
- 2. Harvest the bacterial cells by centrifugation at 6,000 x g for 15 minutes.**
- 3. Equilibrate Maxi Ultraflow™ Columns by applying 5 ml of 98% ethanol. Allow the column to empty by gravity flow and discard the filtrate.**
- 4. Apply 10 ml of VPN Buffer to the Maxi Ultraflow™ Column and allow it to flow 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.**

The volume of added E² Reagent should be more than 1/10 volume of the supernatant from Step. 8.

- 10. Apply the mixture to the Maxi Ultraflow™ Column and allow it to flow through by gravity flow and discard the filtrate.**
- 11. Wash the column twice with 30 ml of VPN Buffer by gravity flow and discard the filtrate.**
- 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.)**
- 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.**