
User Bulletin
GMV1002

Cat. #:

Maxi-V500™ Ultrapure Plasmid Extraction System

Viogene Maxi-V500™ Ultrapure Plasmid Extraction System allows the isolation of ultrapure plasmid DNA from up to 250 ml culture. Plasmid DNA purified from Viogene's proprietary anion-exchange resin is suited for use in transfection, automated sequencing and enzymatic modification.

Kit contents:

VP1 Buffer (1), VP2 Buffer (1), VP3 Buffer (1), VP4 Buffer (2), VP5 Buffer (3), VP6 Buffer (1), RNase A (1), Maxi-V500™ Column (25), Mini-M™ Column (50) and protocol (1)

Notes:

Please read the following notes before starting the procedures.

- Spin RNase A solution tube before use, add 1 ml of VP1 Buffer and vortex to mix well. Apply the mixture into VP1 Buffer bottle and store at 4°C.
- If precipitation forms in VP2 Buffer, incubate at 55°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.

Protocol:

- 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 12-18 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-V500™ Columns by applying 20 ml of VP4 Buffer. Allow the column to empty by gravity flow and discard the filtrate.**
- 4. Resuspend the cell pellet in 10 ml of VP1 Buffer.**
The bacterial cells should be completely resuspended before adding VP2 Buffer.
- 5. 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.
- 6. Add 10 ml of ice-cold VP3 Buffer, mix gently by rotating.**
After adding VP3 Buffer, white precipitate should be formed.
- 7. 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.
- 8. Apply the supernatant to the Maxi-V500™ Column and allow it to flow through by gravity flow and discard the filtrate.**

9. Wash the column once with 30 ml of VP5 Buffer by gravity flow and discard the filtrate.
10. Apply 10 ml of VP6 Buffer to elute DNA by gravity flow.
11. 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.
12. Wash the DNA pellet with 5 ml of room temperature 70 % ethanol and centrifuge at 15,000 x g for 10 minutes. Carefully remove the supernatant.
13. Air-dry the DNA pellet for 10 minutes and dissolve the DNA in 300 μ l or a suitable volume of TE or ddH₂O.
14. Some insoluble material may also elute out from the column at step 10. To eliminate the insoluble material, load the dissolved DNA sample into a Mini-MTM 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.
15. Store DNA at -20°C .