

User Bulletin

Cat. #: GDV1002

Midi-V100™ Ultrapure Plasmid Extraction System

Viogene Midi-V100™ Ultrapure Plasmid Extraction System allows the isolation of ultrapure plasmid DNA from up to 50 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), Midi-V100™ Column (50), Mini-M™ Column (100) 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 25-50 ml (high-copy-number plasmids) or 50-100 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 Midi-V100™ Columns by applying 10 ml of VP4 Buffer. Allow the column to empty by gravity flow and discard the filtrate.**
4. **Resuspend the cell pellet in 4 ml of VP1 Buffer.**
The bacterial cells should be completely resuspended before adding VP2 Buffer.
5. **Add 4 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 4 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 Midi-V100™ Column and allow it to flow through by gravity flow and discard the filtrate.**

9. Wash the column once with 15 ml of VP5 Buffer by gravity flow and discard the filtrate.
10. Apply 5 ml of VP6 Buffer to elute DNA by gravity flow.
11. Precipitate DNA by adding 3.75 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 100 µl 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.