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VIOGENE

User Bulletin

Endotoxin-Free Ultrapure Plasmid Extraction Midiprep System

Viogene Endotoxin-Free Ultrapure Plasmid Extraction Midiprep System allows the isolation of ultrapure and endotoxin-free plasmid DNA from up to 100 ml E. *Coli* culture. The suggested volume of VP1-3 Buffer used in the protocol is developed for 50ml sample culture. If starting sample culture is larger than 50 ml, please increase the volume of VP1, 2, and 3 Buffer proportionally.

Downstream Application

* Mammalian cell transfection
* Gene silencing study
* Ligation and cloning
* Sequancing
* *In vitro* transcription

Product Contents

Cat. No	GDN1001
Preps	25
VP1 Buffer	120 ml
VP2 Buffer	120 ml
VP3 Buffer	120 ml
VPN Buffer	265 ml *4
VPE Buffer	150 ml
E^2 Reagent	10 ml
RNase A (20mg/ml)	0.6 ml
Mini *Plus* Column	50
Midi Ultraflow Column	25
Protocol	1

All buffers need to be mixed well before use.

Shipping & Storage

Viogene Endotoxin-Free Ultrapure Plasmid Extraction Midiprep System is shipping and storage at ambient temperature up to 12 months. For the best result, please storage VP1 and VP3 buffer at 4℃ upon receiving.

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℃.
- If precipitation forms in VP2 Buffer, incubate at 55℃ for 10 minutes to redissolve the salt precipitate. Do not shake VP2 Buffer, SDS present will lead to serious foaming.
- 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 provided in the kit (e.g. isopropanol, 70% ethanol, and TE buffer) should be endotoxin-free grade and freshly prepared with endotoxin-free water (ex. Pure water from Milli-Q Biocel System or Synthesis System from Millipore® with Pyroguard® cartridge).
- Add 40 ml Isopropanol into E^2 Reagent bottle and mix completely by inverting several times, when first open.

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 12-16 hours with vigorous shaking at 37℃.**

2. **Harvest the bacterial cells by centrifugation at 6,000 x g for 15 minutes.**

3. **Equilibrate Midi Ultraflow™ Columns by applying 3 ml of 98% ethanol. Allow the column to empty by gravity flow and discard the filtrate.**

4. **Apply 5 ml of VPN Buffer to the Midi Ultraflow™ Column and allow it to flow through by gravity flow and discard the filtrate.**

5. **Resuspend the cell pellet in 4 ml of VP1 Buffer.**
   The bacterial cells should be completely resuspended before adding VP2 Buffer.

6. **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.

7. **Add 4 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℃.**
   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 1.5 ml of E^2 Reagent (Isopropanol added). Invert the tube 8~12 times and stand for 2 minutes.**
   The volume of added E^2 Reagent should be more than 1/10 volume of the supernatant from Step. 8.

10. **Apply the mixture to the Midi Ultraflow™ Column and allow it to flow through by gravity flow and discard the filtrate.**

11. **Wash the column twice with 15 ml of VPN Buffer by gravity flow and discard the filtrate.**

12. **Apply 5 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 3.75 ml (0.75 x volumes) of room temperature isopropanol to the elute. Mix and centrifuge at 15,000 x g for 30 minutes at 4℃. 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 100 µl or a suitable volume of endotoxin-free TE or ddH_2O.**

16. **<Optional> 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℃.**