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Product Data Sheet

Cat # V-210-25	ULTRAPURE PLASMID EXTRACTION SYSTEM	**Size:** 25 Preps
Cat # V-210-50	ULTRAPURE PLASMID EXTRACTION SYSTEM	**Size:** 50 Preps

Ultrapure Plasmid Extraction System

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.

Downstream Application

* Transfection
* Transformation
* Ligation and cloning
* Sequancing
* *In vitro* transcription

Product Contents

Kit # V-210-25 (25 preps)

Kit component	Amounts
Buffer 1 (RNase A added) #210-25-1	10 ml
Buffer 2 #210-25-2	10 ml
Buffer 3 #210-25-3	10 ml
Buffer 4 #210-25-4	25 ml
Buffer 5 #210-25-5	35 ml
Buffer 6 #210-25-6	12 ml

Kit # V-210-50 (50 preps)

Kit component	Amounts
Buffer 1 (RNase A added) #210-25-1	10 ml
Buffer 2 #210-25-2	10 ml
Buffer 3 #210-25-3	10 ml
Buffer 4 #210-25-4	25 ml
Buffer 5 #210-25-5	35 ml
Buffer 6 #210-25-6	12 ml

Cat. No	**GDV0000**
Preps	2
VP1 Buffer (RNase A added)	10ml
VP2 Buffer	10ml
VP3 Buffer	10ml
VP4 Buffer	25ml
VP5 Buffer	35ml
VP6 Buffer	12ml
Mini-M Column	4
Midi-V100 Column	2
Protocol	1

Shipping & Storage

The sample of Midi-V100™ Ultrapure Plasmid Extraction System is shipping and storage at ambient temperature up to 6 months.

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

Protocol

❖ **Please read the following notes before starting the procedures.**

Important Notes

- 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.
- Sit VP3 Buffer on ice before use.
- The volume of VP1-3 Buffer used in the protocol is developed for 50ml sample culture. If starting sample culture is larger than 50ml, please increase the volume of VP1-3 buffer proportionally.

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-V100™ Columns by applying 3 ml of 98% ethanol. Allow the column to empty by gravity flow and discard the filtrate.**

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4. **Apply 10 ml of VP4 Buffer to the Midi-V100™ 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. **Apply the supernatant to the Midi-V100™ Column and allow it to flow through by gravity flow and discard the filtrate.**

10. **Wash the column once with 15 ml of VP5 Buffer by gravity flow and discard the filtrate.**

11. **Apply 5 ml of VP6 Buffer to elute DNA by gravity flow.**

12. **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□. Carefully remove the supernatant.**

13. **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.**

14. **Air-dry the DNA pellet for 10 minutes and dissolve the DNA in 100 µl or a suitable volume of TE or ddH_2O.**

15. **Some insoluble materials may also elute out from the column at step 10. To eliminate the insoluble material, load the dissolved DNA sample into a Mini-M™ 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.**

16. **Store DNA at -20□.**

All buffers need to be mixed well before use.