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Version: 01

mi-Plasmid Midiprep Kit

Cat. No. mi-PMD25 & mi-PMD50

metabion's mi-Plasmid Midiprep Kit allows the isolation of ultrapure plasmid DNA from up to 50ml culture. Plasmid DNA purified from ***metabion***'s proprietary anion-exchange resin is suited for use in transfection, automated sequencing and enzymatic modification.

Downstream Applications

- Transfection
- Transformation
- Ligation and cloning
- Sequencing
- *In vitro* transcription

Product Contents

Cat. No.	mi-PMD25	mi-PMD50
Preps	**25**	**50**
VP1 Buffer	120ml	265ml
VP2 Buffer	120ml	265ml
VP3 Buffer	120ml	265ml
VPN Buffer	265ml x 2	265ml x 4
VPE Buffer	130ml	265ml
RNase A (20mg/ml)	0.600ml	1.325ml
Mini-M Column	50	100
Midi-Column	25	50

Storage Conditions

metabion's mi-Plasmid Midiprep Kit can be stored at room temperature up to 12 months. If precipitate forms in any buffer, incubate at 37°C for 30 minutes to resuspend. RNAse A is preferentially stored at 4°C.

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Protocol

Please read the following notes before starting the procedures.

Important Notes

- All buffers need to be mixed well before use!
- Spin RNase A solution tube before use; add all of RNase A solution into VP1 Buffer bottle and mix well; store at 4°C.
- If precipitation has formed in VP2 Buffer, incubate at 55°C for 10min (alternatively 30min at 37°C) to resuspend. Do not shake VP2 Buffer, SDS present will lead to serious foaming.
- Sit VP3 Buffer on ice before use.
- The volume of buffers VP1-3 used in the protocol is developed for 50-ml sample culture. If starting sample culture is larger than 50ml, please increase the volume of buffers VP1-3 proportionally.

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Protocol

1. **Culture plasmid-containing bacterial cells in 25-50ml (high-copy-number plasmids) and 100-250ml (low-copy-number plasmids) of LB medium, respectively. 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 Midi-Columns by applying 3ml of 98% ethanol. Allow the column to empty by gravity flow and discard the filtrate.**

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

5. **Resuspend the cell pellet in 4ml of VP1 Buffer (make sure RNase A has been added!).**
   The bacterial cells should be completely resuspended before adding VP2 Buffer.

6. **Add 4ml of VP2 Buffer, mix gently by inverting the tube and let sit for 5 minutes.**
   Do not vortex; vortexing will shear genomic DNA. The lysate should be clear and viscous.

7. **Add 4ml of ice-cold VP3 Buffer, mix gently by rotating.**
   After adding VP3 Buffer, white precipitate should form.

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. **Apply the supernatant to the Midi-Column, allow it to flow through by gravity and discard the filtrate.**

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

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

12. **Precipitate DNA by adding 3.75ml (0.75 volumes) of room temperature isopropanol to the eluate. Mix and centrifuge at 15,000 x g for 30 minutes at 4°C. Carefully remove the supernatant.**

13. **Wash the DNA pellet with 5ml 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 100µl or a suitable volume of TE or ddH_2O.**

15. **Some insoluble material may also elute from the column at step 11. To eliminate the insoluble material, load the dissolved DNA sample onto 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. **Short-term (up to a few weeks), store at 4°C; long-term, store at -20°C. To avoid multiple freeze-thaw cycles, preparation of aliquots is recommended.**