metabion international AG
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Version: 01

mi-Plasmid Maxiprep Kit

Cat. No. mi-PMX10 & mi-PMX25

metabion's mi-Plasmid Maxiprep Kit allows the isolation of ultrapure plasmid DNA from up to 250ml 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-PMX10	mi-PMX25
Preps	10	25
VP1 Buffer	120ml	265ml
VP2 Buffer	120ml	265ml
VP3 Buffer	120ml	265ml
VPN Buffer	225ml x 2	265ml x 4
VPE Buffer	120ml	265ml
RNase A (20mg/ml)	0.600ml	1.325ml
Mini-M Column	20	50
Maxi-Column	10	25
Manual	1	1

Storage Conditions

metabion's mi-Plasmid Maxiprep 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, apply all of RNase A solution into VP1 Buffer bottle and mix well; store at 4°C.
- If precipitation forms in VP2 Buffer, incubate at 55°C for 10 minutes to re-dissolve.
- Do not shake VP2 Buffer, SDS present will lead to serious foaming.
- Sit VP3 Buffer on ice before use.
- After the centrifugation step of alkaline lysis, passing the lysate through a folded filter is recommended. The filter will remove SDS and cellular debris from the lysate, and shorten the column flow-through time of the sample filtration. After filtration, continue to step 9 of the protocol.
- The volume of buffers VP1-3 used in the protocol is developed for 100-ml sample culture. If starting sample culture is larger than 100ml, please increase the volume of buffers VP1-3 proportionally.

Ratio of Maxi Plus™ Buffers (ml)					
Bacterial sample volume	***100***	***200***	***300***	***400***	***500***
VP1 Buffer	10	20	30	40	50
VP2 Buffer	10	20	30	40	50
VP3 Buffer	10	20	30	40	50
VPN Buffer	30	30	30	30	30
VPE Buffer	10	10	10	10	10
Isopropanol	7	7	7	7	7
70%ethanol	10	10	10	10	10

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Protocol

1. **Culture plasmid-containing bacterial cell in 100-250ml (high-copy-number plasmids) or 350-500ml (low-copy-number plasmids) of LB medium. 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 Maxi-Columns by applying 5ml of 98% ethanol. Allow the column to empty by gravity flow and discard the filtrate.**

4. **Apply 10ml of VPN Buffer to the Maxi-Column and allow it to flow through by gravity and discard the filtrate.**

5. **Resuspend the cell pellet in 10ml of VP1 Buffer (make sure RNase A has been added!).**
   The bacterial cells should be completely resuspended (no cell clump should be visible!) before adding VP2 Buffer.

6. **Add 10ml of VP2 Buffer, mix gently by rotating the lysate and stand for 5 minutes.**
   Do not vortex; vortexing will shear genomic DNA contaminating plasmids, and cause unwanted foaming. The lysate should be clear and viscous.

7. **Add 10ml 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.**
   Passing the supernatant lysate through a folded filter is recommended (see *Important Notes, page 3*) before proceeding to step 9.

9. **Apply the supernatant to the Maxi-Column, allow it to empty by gravity flow and discard the filtrate.**

10. **Wash the column once with 30ml of VPN Buffer by gravity flow and discard the filtrate.**

11. **Apply 10ml of VPE Buffer to elute DNA by gravity flow.**

12. **Precipitate DNA by adding 7.5ml (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 250µl 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.**