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Version: 01

mi-Plasmid Miniprep Kit

Cat. No. mi-PMN50 & mi-PMN250

metabion's mi-Plasmid Miniprep Kit provides a simple, fast and cost-effective method to purify plasmid DNA without phenol/chloroform extraction. It is based on binding DNA to silica-based membranes in chaotropic salts. An average yield of 8-40µg of plasmid DNA can be expected from 1-5ml overnight bacterial culture.

Downstream Applications

- Restriction digestion
- Ligation and cloning
- PCR & Sequencing
- Transformation
- Transfection
- In vitro transcription
- …

Product Contents

Cat. No.	mi-PMN50	mi-PMN250
Preps	**50**	**250**
MX1 Buffer	12ml	60ml
MX2 Buffer	15ml	75ml
MX3 Buffer	20ml	100ml
WN Buffer	6ml	30ml
WS Buffer	10ml	45ml
Elution Buffer	5ml	25ml
RNase A (20mg/ml)	0.042ml	0.210ml
Mini Column	50	250
Collection Tube	50	250

Storage Conditions

metabion's mi-Plasmid Miniprep 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.

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 into the MX1 Buffer and mix well; store at 4°C.
- Buffers provided in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn.
- If precipitation has formed in MX2 Buffer, incubate at 55°C for 10 minutes to redissolve.
- Do not shake MX2 Buffer, SDS present will lead to serious foaming.
- All procedures should be done at room temperature (20-25°C).
- All centrifugation steps are done at 7,000 x g - 10,000 x g (9,000-13,000 rpm) in a microcentrifuge, unless noted otherwise.
- For long-term storage of the eluted plasmid, TE buffer should be used for elution. Since EDTA in TE may affect downstream applications, Elution Buffer (provided) or ddH_2O (pH 7.0-8.5) is preferred for elution of DNA immediately used for further enzymatic reactions.

For mi-PMN50

- Add 24ml of 98-100% ethanol into WN Buffer bottle when first opened.
- Add 40ml of 98-100% ethanol into WS Buffer bottle when first opened.

For mi-PMN250

- Add 120ml of 98-100% ethanol into WN Buffer bottle when first opened.
- Add 180ml of 98-100% ethanol into WS Buffer bottle when first opened.

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I. DNA Purification by Centrifugation

1. **Grow 1-5ml plasmid-containing bacterial cells in LB medium with appropriate antibiotic(s) overnight (12-16 hours) with vigorous shaking.**

2. **Pellet the cells by centrifuging for 1-2 minutes. Decant the supernatant and remove all residual medium with a pipet.**

3. **Add 200µl of MX1 Buffer (make sure RNase A has been added!) to the pellet, resuspend the cells completely by vortexing or pipetting.**
   No cell clumps should be visible after resuspending the pellet.

4. **Add 250µl of MX2 Buffer and mix gently (invert the tube 4-6 times) to lyse the cells until the lysate becomes clear. Incubate at room temperature for 1-5 minutes.**
   Do not vortex; vortexing will shear genomic DNA.

5. **Add 350µl of MX3 Buffer to neutralize the lysate, then immediately and gently mix the solution.**
   White precipitate should be formed.

6. **Centrifuge at 10,000 x g (13,000 rpm) for 5-10 minutes, meanwhile place a Mini Column onto a Collection Tube.**

7. **Transfer the supernatant carefully into the column.**

8. **Centrifuge at 7,000 x g (9,000 rpm) for 30-60 seconds. Discard the flow-through.**

9. **Wash the column once with 0.5ml WN Buffer by centrifuging at 7,000 x g (9,000 rpm) for 30-60 seconds. Discard the flow-through.**

10. **Wash the column once with 0.7ml WS Buffer by centrifuging at 7,000 x g (9,000 rpm) for 30-60 seconds. Discard the flow-through.**

11. **Centrifuge the column at 10,000 x g (13,000 rpm) for another 3 minutes to remove residual ethanol.**
    It is important to remove residual ethanol since it may inhibit subsequent enzymatic reactions.

12. **Place the column onto a new 1.5-ml centrifuge tube. Add 50µl of Elution Buffer (provided) onto the center of the membrane.**
    For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.

13. **Stand the column for 2-3 minutes and centrifuge at 10,000 x g (13,000 rpm) for 2-3 minutes to elute DNA.**

14. **Short-term (up to a few weeks), store DNA at 4°C; long-term, store at -20°C. To avoid multiple freeze-thaw cycles, preparation of aliquots is recommended.**

II. DNA Purification by Vacuum

1. **Grow 1-5ml plasmid-containing bacterial cells in LB medium with appropriate antibiotic(s) overnight (12-16 hours) with vigorous agitation.**

2. **Pellet the cells by centrifuging for 1-2 minutes. Decant the supernatant and remove all residual medium with a pipet.**

   Add 200µl of MX1 Buffer (make sure RNase A has been added!) to the pellet, resuspend the cells completely by vortexing or pipetting.
   No cell clumps should be visible after resuspending the pellet.

3. **Add 250µl of MX2 Buffer and mix gently (invert the tube 4-6 times) to lyse the cells until the lysate becomes clear. Incubate at room temperature for 1-5 minutes.**
   Do not vortex; vortexing will shear genomic DNA.

4. **Add 350µl of MX3 Buffer to neutralize the lysate, then immediately and gently mix the solution.**
   White precipitate should be formed.

5. **Centrifuge at 10,000 x g (13,000 rpm) for 5-10 minutes, meanwhile insert the tip of a Mini Column into the luer-lock of a vacuum manifold (e.g. Promega's Vac-man).**

6. **Transfer the supernatant carefully onto the column.**

7. **Apply vacuum to draw all the liquid into the manifold.**

8. **Wash the column once with 0.5ml WN Buffer by re-applying vacuum to draw all the liquid.**

9. **Wash the column once with 0.7ml WS Buffer by re-applying vacuum to draw all the liquid.**

10. **Place the column onto a Collection Tube. Centrifuge the column at 10,000 x g (13,000 rpm) for another 3 minutes to remove residual ethanol.**
    It is important to remove residual ethanol since it may inhibit subsequent enzymatic reactions.

11. **Place the column onto a new 1.5-ml centrifuge tube. Add 50µl of Elution Buffer (provided) onto the center of the membrane.**
    For effective elution, make sure that the solution is dispensed onto the center of the membrane and is completely absorbed.

12. **Stand the column for 2-3 minutes and centrifuge at 10,000 x g (13,000 rpm) for 2-3 minutes to elute DNA.**

13. **Short-term (up to a few weeks), store DNA at 4°C; long-term, store at -20°C. To avoid multiple freeze-thaw cycles, preparation of aliquots is recommended.**