



mi-Plasmid HTP Prep Kit

Cat. no:

mi-PMHTP10 [10x 96 preparations]

mi-PMHTP30 [30x 96 preparations]

(mi-PMHTP3 [3x 96 preparations] Testkit)

**This kit is for research purposes only.
Not for use in diagnostic analysis.
For *in vitro* use only.**

A. Introduction

The metaBION Plasmid HTP Prep Kit is suitable for the isolation of any plasmid DNA from small-scale bacterial cell cultures. This kit is designed for high-throughput plasmid DNA minipreps using 96 cleaning/binding plates on a vacuum manifold or centrifuge. The metaBION PMHTP procedure is based on the alkaline lysis method; exposure of bacterial cells to the strong anionic detergent leads to degradation of cell walls at high pH; plasmid DNA is released into the supernatant. Subsequently, under the high salt condition, plasmid DNA binds to the silica-gel-membrane in the column. Salts and debris are removed in a wash step and finally bound plasmid DNA is eluted by using low salt buffer. Purified plasmid DNA can be utilized for cloning, PCR, sequencing, labeling of probes, cell transfection, electroporation and enzymatic assays.

B. Kit contents

Reagents for	10 plates	30 plates	3 plates (Testkit)
Cell Resuspension Solution	150 ml	500 ml	50 ml
Cell Lysis Solution	150 ml	500 ml	50 ml
DNA Binding Solution	250 ml	800 ml	80 ml
Column Wash Solution	2x 100 ml (add 400 ml EtOH each)	2x 200 ml (add 800 ml EtOH each)	50 ml (add 200 ml EtOH)
RNase A Powder	8,25 mg	27,5 mg	not provided, 2,75 mg needed
96 well Cleaning Filter	10 plates	30 plates	3 plates
96 well Binding Filter	10 plates	30 plates	3 plates
96 well Collection Plates	10 plates	30 plates	3 plates
Sealing Film	10	30	3

C. Required Equipment

- Vortex or plate shaker
- Sterile 96 deep well plates
- Nuclease-free water (pH 7-8) or 10 mM Tris-HCl (pH 8.0)
- Vacuum manifold or Plate centrifuge

D. Storage conditions

The metaBION PMHTP Kit should be stored at room temperature. After addition of RNase A powder, Cell Resuspension Solution must be stored at 4 °C to be stable for 6 months. If the limit time is exceeded, add additional RNase A. Cell Lysis Solution contains the detergent SDS, that can precipitate under cold conditions. The precipitate affects the yield of the DNA. Please completely resuspend SDS by warming up the solution. Under these conditions, the kit is stable for at least 6 months following delivery.

E. Quality control

Manufacturing of all components is performed under clean conditions. Delivered kits are quality controlled by tests like restriction enzyme assay, spectrophotometric analysis and PCR analysis.



F. Detailed protocol

Precautions:

- **Wear gloves to avoid contact with all reagents.**
- **< If eye or skin contact occurs, wash thoroughly with water >**
- **Avoid direct contact of DNA Binding Solution with bleach or other oxidizers.**
- **Warning: Column Wash Buffer is flammable!**

Note: Before starting, please make sure....

- to have completed the “Column Wash Solution” by adding pure ethanol (99.9 %) to each of the two bottles before first use.
- to have completed the “Cell Resuspension Solution” by adding the RNase powder (after addition store the solution at 4 °C).
- to label the plates with an ethanol resistant marker pen.
- to check the “Cell Lysis Solution”. If there are precipitates, heat to 55 ° - 65 °C for 5 minutes to dissolve the SDS completely.

Both protocols consistently deliver 3-4 µg of pGem3Zf plasmid DNA (at 70-90 ng/ul) from *E. coli JM109*.

Version 1 [using a vacuum manifold]

- 1. Inoculate *E.coli* into 1 ml aliquots of LB or 2X LB containing the appropriate antibiotic on sterile 96 deep well plate (2 ml capacity).** Cover plates with lid. Incubate at 37 °C at 250-320 rpm for 20 to 24 hours (Optical density at 650 nm should be approximately 1.3).
- 2. Centrifuge at 1,500x g for 10 min. After centrifugation, immediately decant culture media.** Invert and tap the plates firmly on several layers of paper towels on the bench to remove residual culture media.
- 3. Resuspend pellets in 100 µl of Cell Resuspension Solution using a vortex or a plate shaker.** Complete resuspension is important.
- 4. Add 100 µl of Cell Lysis Solution. Mix immediately and vigorously with a plate shaker or vortex for 1 min. Incubate for additional 2 min at room temperature.** Do not exceed exposure to Cell Lysis Solution for more than 5 min or plasmid DNA may denature irreversibly.
- 5. Add 170 µl of DNA Binding Solution. Mix immediately and vigorously with a plate shaker or vortex for 2 min.**

- 6. To remove the debris, slowly pipett 175 µl of the lysate from the bottom of each deep well and dispense it into the corresponding well of a Cleaning Plate.
Repeat this step: Transfer another 175 µl from the same well into the Cleaning plate.**
- 7. Place the Binding Plate in the bottom of the vacuum manifold (Millipore: MAVM096 or equivalent).**
- 8. Place the Cleaning Plate on top of the Binding Plate, and adjust the vacuum to 250 mbar. Do not exceed 250 mbar vacuum setting during filtration of the lysate to ensure a uniform filtration.**
- 9. Apply the vacuum for 5-10 min, drawing the lysate through the Cleaning Plate into the Binding Plate (until wells are empty). Discard the Cleaning Plate.**
- 10. Place the Binding Plate on top of the empty manifold. Apply full vacuum for 1-2 min (or until the wells are empty). Plasmid DNA is now bound to the Binding Plate.**
- 11. Add 300 µl of Wash Solution to each well of the Binding Plate. Apply full vacuum for 1 min.**
- 12. Repeat <Step 11>, but apply vacuum for 3 min (until the wells are empty).**
- 13. Remove the Binding Plate from the manifold. Tap the plates firmly on several layers of paper towels on the bench to remove residual alcohol.**
- 14. Place the Binding Plate on top of deep well plate and apply the vacuum for 5-10 min to dry. Alternatively, the plate can be dried at 60 °C for 30 min.**
- 15. Place the Binding Plate on top of a microtiter plate. Apply 50-100 µl of deionized water or 10 mM Tris-HCl, pH 8.0 (not provided) directly in the middle of the Binding Plate membrane. Let stand for 1 min and elute by vacuum or centrifuge at 1000x g for 5 minutes.**



Version 2 [using a centrifuge]

- 1. Inoculate *E.coli* into 1 ml aliquots of LB or 2X LB containing the appropriate antibiotic on sterile 96 deep well plate (2 ml capacity).** Cover plates with lid. Incubate at 37 °C at 250-320 rpm for 20 to 24 hours (Optical density at 650 nm should be approximately 1.3).
- 2. Centrifuge at 1,500x g for 10 min. After centrifugation, immediately decant culture media.** Invert and tap the plates firmly on several layers of paper towels on the bench to remove residual culture media.
- 3. Resuspend pellets in 100 µl of Cell Resuspension Solution using a vortex or a plate shaker.** Complete resuspension is important.
- 4. Add 100 µl of Cell Lysis Solution. Mix immediately and vigorously with plate shaker or vortex for 1 min. Incubate for additional 2 min at room temperature.** Do not exceed exposure to Cell Lysis Solution for more than 5 min or plasmid DNA may denature irreversibly.
- 5. Add 170 µl of DNA Binding Solution. Mix immediately and vigorously with plate shaker or vortex for 2 min.**
- 6. To remove the debris, slowly pipett 175 µl of the lysate from the bottom of each deep well and dispense it into the corresponding well of a Cleaning Plate.**
Repeat this step: Transfer another 175 µl from the same well into the Cleaning plate.
- 7. Place the Cleaning Plate on top of a Binding Plate.**
- 8. Place the set prepared <Step 7> on top of a deep well plate.**
- 9. Centrifuge at 2,000x g for 20 min. Discard the Cleaning Plate and the waste in deep well plate.**
- 10. Place the Binding Plate on top of the deep well plate. Add 300 µl of Wash Solution to each well of the Binding Plate and centrifuge at 2,000x g for 5 min. Discard the waste in deep well plate.**
- 11. Repeat <Step 10>, but centrifuge for 10 min.**
- 12. Place the Binding Plate on top of a microtiter plate. Apply 50-100 µl of deionized water or 10 mM Tris-HCl, pH 8.0 (not provided) directly in the middle of the Binding Plate membrane. Let stand for 1 min and centrifuge at 1,000x g for 5 minutes or elute by vacuum.**

G. Hints and troubleshooting

Symptoms	Possible Causes	Comments
Low yield of plasmid DNA	Resuspension of bacterial pellet is not complete	Cells trapped in clumps will be resistant to lysis reagents. Clumps should be thoroughly resuspended in Resuspension Solution.
	Lysis Solution precipitated	Cell Lysis Solution contains the detergent SDS, that can precipitate under cold conditions. The precipitate is resuspended easily by heating.
	Poor lysis of resuspended cells	Incomplete lysis reduces chance in exposure of DNA. Ensure complete mixing.
	Unsuitable Elution buffer	Elution efficiency is dependent on pH and salt concentration. The optimal efficiency is obtained with a pH 7.0-8.5 and in the presence of low-salt buffer (e.g. 10 mM Tris-HCl, pH 8.0 or deionized water)
	Unsuitable cell volume	Maximum volumes are 5 ml for high copy plasmids and 10 ml for low copy plasmids. Don't use more than 2 ml of rich culture media such as TB and 2x YT.
Poor enzymatic reaction	Too high salt concentration in eluate	The common choice is repetition of the wash step. Alternatively, let the binding plate for 5 min at room temperature after adding Wash Solution.
Chromosomal DNA or RNA contamination	Misappropriated lysis and neutralization step	Lysis step must not exceed 5 min. Too vigorous mixing can shear chromosomal DNA. After addition of neutralization buffer, mix immediately.
	Over the limit time of RNase and inadequate storage	After addition of RNase A to the Resuspension Solution, solution must be stored at 4 °C. If the limit time (6 month) is exceeded, add additional RNase A.
Degraded DNA	Culture overgrown	Don't let cultures grow longer than 24 hours. Overgrown cultures lead to degraded DNA (also plasmids)