mi-PCR Purification HTP Kit

Cat. no:
mi-PPHTP10 [10x 96 preparations]
mi-PPHTP30 [30x 96 preparations]

(mi-PPHTP3 [3x 96 preparations] Testkit)

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

metabion international AG – Lena-Christ-Str. 44/I – 82152 Martinsried – Tel: +49 89 899 363 0 – Fax: +49 89 899 363 11

http://www.mymetabion.com - e-mail orders to: mi@mymetabion.com
quality…value…service…www.mymetabion.com

A. Introduction

The metaBIOn PCR Purification HTP Kit is suitable for high-throughput PCR purifications using 96 well binding plates on a vacuum manifold or centrifuge. DNA Binding Solution is added to the PCR products and the mixtures are applied to 96 well binding plates containing glass fiber-based membranes where the double stranded DNA is selectively absorbed. DNA polymerase, buffer, unreacted primers, and dNTP are removed with the alcohol-containing Column Wash Solution. Since the DNA is eluted with nuclease-free water or Tris-buffer, precipitation is not necessary. This kit eliminates the use of organic solvent such as phenol and chloroform. The recovery of isolated DNA fragments, which are between 100 bp and 10 kb in length can be obtained at least 70 %, and used directly for sequencing, cloning, and any kinds of enzymatic reactions.

B. Kit contents

Reagents for	**10 plates**	**30 plates**	3 plates (Testkit)
DNA Binding Solution	350 ml	2x 500 ml	100 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)
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
Nuclease-free water (pH 7-8) or 10 mM Tris-HCl (pH 8.0)
Vacuum manifold or Plate centrifuge

D. Storage conditions

The metaBIOn PPHTP Kit should be stored at room temperature. The DNA Binding 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 label the plates with an ethanol resistant marker pen.
- to check the "DNA Binding Solution". If there are precipitates, heat to 55 ° - 65 °C for 5 minutes to dissolve the SDS completely.

Version 1 [using a vacuum manifold]

1. **Add 5 volumes of DNA Binding Buffer to your PCR reaction and mix well.** For example, add 250 µl to 50 µl PCR reaction. Mix immediately and vigorously (maximum speed) using a plate shaker for 2 min.

2. **Place a Binding Plate on top of the manifold, and adjust the vacuum to 250 mbar.** Do not excess 250 mbar vacuum setting during filtration of the mixtures to ensure uniform filtration.

3. **Transfer the DNA/Binding Solution mixtures into the binding plate.**

4. **Apply the vacuum for 5-10 min, drawing the solution through the Binding Plate.** The DNA is now bound to the Binding Plate.

5. **Add 300 µl of Wash Buffer to each well of the Binding Plate.** Apply full vacuum for 1 min.

6. **Repeat step 5, but apply vacuum for 3 min.**

7. **Remove the Binding Plate from the manifold.** Tap the plates firmly on several layers of paper towels on the bench to remove residual alcohol.

8. **Apply the vacuum for 3 min to remove residual alcohol.**

9. Place the Binding plate on top of a collection plate. Apply 50 to 100µl of deionized water or 10mM Tris-HCl, pH 8.0 (not provided) directly in the middle of the Binding Plate membrane. Let stand for 1min. Maximum recovery is obtained with nuclease-free water warmed to 60-70 °C.

10. Apply the vacuum for 5-10 min.

Version 2 [using a centrifuge]

1. Add 5 volumes of DNA Binding Buffer to your PCR reaction and mix well. For example, add 250 µl to 50 µl PCR reaction. Mix immediately and vigorously (maximum speed) using a plate shaker for 2 min.

2. Place a Binding Plate on top of a deep well plate.

3. Transfer the DNA/Binding Solution mixtures into the binding plate.

4. Centrifuge at 2000x g for 5 min. Discard the flow-through and replace the binding filter on the deep well plate.

5. Add 300 µl of Wash Buffer to each well of the Binding plate.

6. Centrifuge at 2000x g for 5 min. Discard the waste in deep well plate and replace the Binding plate on a deep well plate.

7. Repeat step 5 and 6, but centrifuge for 10 min.

8. Tap the plates firmly on several layers of paper towels on the bench to remove residual alcohol. Centrifuge at 2000x g for 5 min to remove residual alcohol.

9. Place the Binding plate on top of a collection tube. Apply 50 to 100 µl of deionised 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. Maximum recovery is obtained with nuclease-free water warmed to 60-70 °C.

10. Centrifuge at 2000x g for 5 min.

G. Hints and troubleshooting

Symptoms	Possible Causes	Comments
No or poor DNA yield	Incorrect dispensation of Elution buffer.	Elution buffer should be dispensed to center of the membrane. Increase the elution volume to 200 µl maximum for high efficiency of DNA recovery.
	Unsuitable Elution buffer	Elution efficiency is dependent on pH and salt concentration. The optimal efficiency is obtained at pH 7.0 - 8.5 and in the presence of low-salt buffer (ex. 10 mM Tris-HCl, pH 8.5 or destilled water). To ensure yield, preheat elution buffer to 70 °C before elution. Apply Tris buffer or destilled water directly onto the center of glass fiber membrane.
Poor enzymatic reaction	Too high salt concentration in the eluate	The common choice is the repetition of the washing step. Alternatively, leave the Binding Plate for 5 min. at room temperature after adding Washing Solution.
	Residual Washing Solution in eluate	Centrifuge for an additional 5 min to remove residual Washing Solution.
	Smaller smeared band is appears on analytic gel.	In this case, denatured ssDNA can be contained in the eluate. To reanneal the ssDNA, heat the eluate at 95 °C for 2 min. and allow the tube slowly at room temperature.
Degraded DNA	Sample is too old or mis-stored.	Old and mis-stored samples lead to degraded DNA.