

Micro PCR Purification Kit



User Guide

mdi

Membrane Technologies

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1. Introduction

mdi Micro PCR Purification Kit is a fast, economical and easy way to obtain highly concentrated, purified DNA fragments (70bp-4kb) from PCR amplification reactions. The buffer system provided in the kit allows selective binding of DNA fragments from PCR reactions onto the spin column.

Washing is done with the help of provided wash buffer in order to remove primers, nucleotides, enzymes, mineral oil, salts and other impurities from DNA samples. Purified DNA is eluted in low salt buffer or water for a variety of downstream applications. The technology also does away with the cumbersome methodologies of phenol extraction (associated with slurries formation) as well as ethanol precipitation (associated with anion exchange based purification system) for desalting.

2. Applications

1. Ligation
2. Transformation/ Transduction/ Transfection
3. Automated Fluorescent Sequencing
4. Radioactive Sequencing
5. Restriction Digestion
6. Labeling
7. Cloning

3. Storage Conditions

As soon as you receive the kit, open it and store **mdi** Micro PCR spin columns at 2-8 °C.

The remaining kit components can be stored at room temperature. The kit is stable for one year at above storage conditions without showing any reduction in performance and quality. If precipitates appear in buffers re-dissolve at 37 °C for few minutes.

4. Quality Assurance

The mdi Micro PCR purification kit is designed for various pre-determined specifications and user requirements such as yield, purity, ruggedness, shelf life and functional convenience.

These are produced through a well defined quality management system certified by Underwriters Laboratories, USA for ISO 9001: 2008 which ensures intra lot as well as lot to lot consistency. The QMS also involves a stringent validated quality control to ensure that all lots being released meet their pre determined specifications.

5. Safety Information

The buffers and the reagents may contain irritants, so wear lab coat, disposable gloves and protective goggles while working with the Micro PCR Purification Kit.

6. Lot Release Criteria

In accordance with mdi ISO-certified quality management system, each lot of Micro PCR Purification Kits is tested against predetermined specifications to ensure consistent product quality.

7. Technical Support

At mdi, customers are our priority. We will share our experiences to assist you to overcome problems in general product usage as well as customize products for special applications. We will

- * Stimulate problems, and suggest alternative methods to solve them.
- * Make changes/ improvements in our existing products/protocols.
- * Develop special new products and system especially to satisfy your needs.

We welcome your feedback to improve our products.

8. Kit Contents

Contents	Quantity			Storage Temperature
	50	250	1000	
Spin Columns	50	250	1000	2-8° C
Buffer MPB	30ml	150ml	600ml	RT
Buffer PW	60ml	300ml	1200ml	RT
Buffer PE	15ml	75ml	300ml	RT
Collection Tubes	50	250	1000	RT
Hand Book	1	1	1	-
Certificate of Quality	1	1	1	-

9. Specifications

Capacity of column reservoir	800µl
Binding capacity of membrane (ds DNA)	5µg
Recovery of DNA	80%
Recovered DNA fragment	(70 bp - 4 kb)
Minimum elution volume	10µl
Total eluate volume	9µl

10. Principle

The **mdi** Micro PCR purification kit allows the isolation of high ultra pure DNA fragments which involves:

1. Capturing PCR amplified DNA on spin column
2. Washing
3. Elution

1. Capturing PCR amplified DNA on spin column

In order to facilitate adsorption of DNA fragments from PCR amplified reactions onto the spin column, optimum salt concentration and pH conditions are necessary which is achieved by addition of binding buffer 'MPB'.

2. Washing

Subsequent to DNA binding onto the spin columns, unwanted components like primers, nucleotides, mineral oil, salts, and other impurities are washed away. Washing is done by buffer 'PW'.

3. Elution

Salt concentration and pH of elution buffer is important for maximum elution efficiency, elution occurs at basic conditions and low salt concentration. Elution is done by buffer 'PE'.

11. Important Points to be Considered

Optimization of Binding Buffers

The binding buffers should possess appropriate salt concentration and pH to facilitate the efficient binding of single or double stranded DNA (70 bp - 4 kb) from PCR reaction on spin column.

Centrifugation

All centrifugation steps should be carried out at room temperature at $\geq 10,000$ rpm in a conventional table top microcentrifuge.

In case of choking of spin column, increase centrifugation time and centrifugation speed to 12,000 rpm.

Washing

To remove residual wash buffer, spin the column for 1-2 minutes at $\geq 10,000$ rpm.

Flow through should be properly discarded before additional centrifugation step otherwise it will not be removed & may inhibit enzymatic reactions.

Elution

Elution buffer should be dispensed on to the center of column for maximum elution efficiency. If eluate volume is lower, then centrifuge for one minute extra at $\geq 10,000$ rpm.

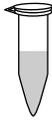
Yield

DNA Yield depends on following factors:

1. Volume of elution buffer
2. Point of application of buffer on the column
3. Incubation period of buffer on the column

12. mdi Micro PCR Purification Procedure

PCR reaction



Add 5 volumes of
buffer MPB



Bind



Wash



Dry



Elute



Ultra Pure
DNA Fragment

13. Protocol

Note: a) All steps should be carried out at room temperature.

b) All centrifugation steps are at $\geq 10,000$ rpm in a conventional table top micro centrifuge.

1. Add 5 volumes of buffer 'MPB' to 1 volume of PCR reaction and mix well. Users may or may not remove mineral oil or kerosene.

For example, add 250 μ l of buffer 'MPB' to 50 μ l PCR reaction (not including oil).

2. Place the spin column in a 2ml collection tube in a suitable rack. Load the above sample mix in the center of the spin column, then centrifuge for 1 min at $\geq 10,000$ rpm and discard the flow through.

Note: Maximum volume of the column reservoir is 800 μ l. For sample volumes $>800\mu$ l, simply load, balance and spin at $\geq 10,000$ rpm for 1min. Discard the flow through.

3. Place the spin column in the same collection tube. Wash the spin column with 750 μ l of buffer 'PW' by giving a spin at $\geq 10,000$ rpm for 1 minute. Discard the flow through.

4. Place the spin column in the same collection tube and centrifuge for 1-2 minutes at $\geq 10,000$ rpm.

Important: This step is necessary to remove residual wash buffer. Residual wash buffer will not be completely removed unless the flow through is discarded before this additional centrifugation. Residual wash buffer may inhibit subsequent enzymatic reactions.

5. Place the spin column in a fresh 1.5ml microcentrifuge tube (not provided). Elute DNA by dispensing 10 μ l of elution buffer 'PE' directly onto the center of the column membrane, allow it to stand for 1 minute and centrifuge for 1 minute at $\geq 10,000$ rpm.

Note: The elution buffer should be dispensed on to the center of the column membrane for maximum recovery. The average eluate volume is 9 μ l from 10 μ l elution buffer. If the eluate volume is lower, then centrifuge for one minute extra at $\geq 10,000$ rpm.

14. Trouble Shooting Guide

This section highlights some of the commonly faced problems, the probable reasons and suggestions to solve them.

Problem #1:	Low or no recovery.
Reason	Elution buffer not properly dispensed.
Solution	The elution buffer must be added to the center of the membrane to ensure that the entire membrane area is covered by it and no elution buffer lies wasted sticking to the sides of the spin column and incubate 2-3 min more before centrifugation.
Problem #2:	Poor quality of recovered DNA.
Reason	Residual wash buffer in the spin column.
Solution	Incomplete drying results in wash buffer in the eluate which is difficult to load in gel and also results in reduced yield and performance in down stream applications. Dry for one minute extra by centrifuging at $\geq 10,000$ rpm to completely remove the wash buffer.

15. Product Use Limitations

mdi kits are developed and manufactured for research purpose only. The products are not recommended to be used for human diagnostic or drug purposes for which these should be cleared by the concerned regulatory bodies in the country of use.

16. Product Warranty and Satisfaction Guarantee

All mdi products are guaranteed and are backed by our

- a. Technical expertise and experience of over 30 years.
- b. Special mdi process for consistency and repeatability.
- c. Strict quality control and quality assurance regimen.
- d. Certificate of analysis accompanied with each product.

mdi provides an unconditional guarantee to replace the kit if it does not perform for any reasons other than misuse. However, the user needs to validate the performance of the kit for its specific use.

17. Ordering Information

To order please specify as below:

Type		XX	XX	XX	X	Pack Size	
Type	Code					Pack Size	Code
MPCK	MPCK					50	0050
						250	0250
						1000	1000

Example:

MPCK	XX	XX	XX	X	1000
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