PCR Purification Kit

User Guide

mdi
Membrane Technologies

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1. Introduction

mdi PCR Purification Kit is a fast, economical and easy way to purify DNA fragments (100bp-10kb) from PCR amplification reactions. The buffer system provided in the kit allows selective binding of DNA fragments from PCR reactions to 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 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

mdi PCR Purification Kit should be stored at room temperature. The kit is stable for one year at above storage conditions without showing any reduction in performance and quality.

For longer storage, the entire kit can be stored at 2-8°C. In case precipitates are observed in buffer, re-dissolve all buffers before use at 37°C for few minutes. All buffers should be at room temperature before starting the protocol.

4. Quality Assurance

The mdi 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.

5. Safety Information

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

6. Lot Release Criteria

Each lot of PCR Purification Kit 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
Spin Columns	50	250	1000	RT
Collection Tubes	50	250	1000	RT
Buffer PB	30ml	150ml	600ml	RT
Buffer PW	60ml	300ml	1200ml	RT
Buffer PE	15ml	75ml	300ml	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)	10µg
Recovery of DNA	90-95%
Recovered DNA fragment	(100 bp - 10 kb)
Minimum elution volume	30µl
Total eluate volume	28µl

10. Principle

Obtaining highly pure DNA fragments using mdi PCR Purification Kit involves: Capturing PCR amplified DNA on spin column, Washing and 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 'PB'.

2. Washing

   Subsequent to DNA binding onto the spin columns, unwanted components like primers, nucleotides, enzymes, 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 with low salt concentration buffer 'PE'.

4. Yield and Concentration

   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

11. Important Points to be Considered

Optimization of Binding Buffers

The binding buffers should posses appropriate salt concentration and pH to facilitate the efficient binding of single or double stranded DNA (100bp-10kb) from PCR reaction on spin column.

Centrifugation

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

In case of choking of spin column, increase centrifugation time.

Washing

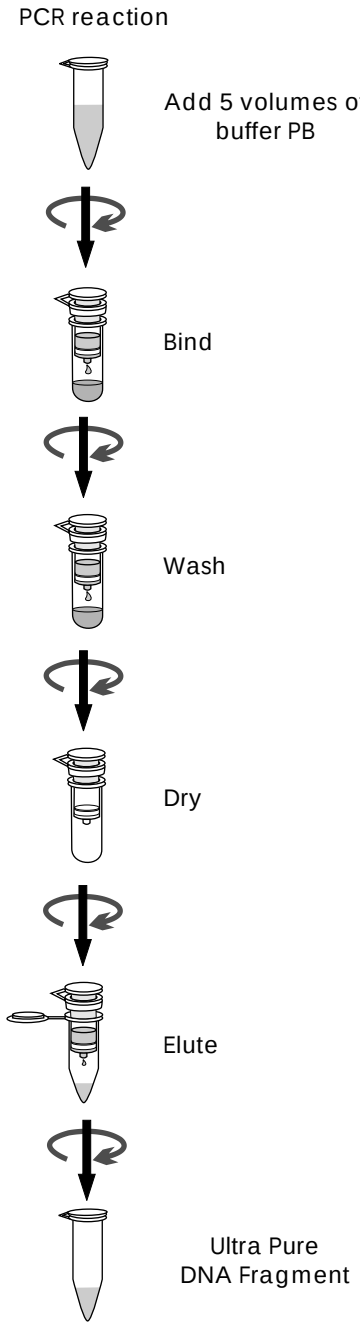
To remove residual wash buffer, spin the column for 1-2 minutes at ≥10,000 rpm.

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 ≥10,000 rpm.

12. mdi PCR Purification Procedure



13. Protocol

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

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

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

   For example, add 250 µl of buffer 'PB' to 50 µl 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 1min at ≥10,000 rpm and discard the flow through.

   Note: Maximum volume of the column reservoir is 800µl. For sample volumes >800µl, simply load, balance and spin at ≥ 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 µl of buffer 'PW' by giving a spin at ≥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 ≥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 30µl 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 ≥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 28µl from 30µl elution buffer. If the eluate volume is lower, then centrifuge for one minute extra at ≥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
SPCK	SPCK					50	0050
						250	0250
						1000	1000

Example:

SPCK	XX	XX	XX	X	0250

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