Express 96 PCR Purification Kit

User Guide

mdi
Membrane Technologies

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1. Introduction

mdi Express 96 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 Express prep plate.

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 Express 96 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 Express 96 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 Express 96 PCR Purification Kit.

6. Lot Release Criteria

Each lot of Express 96 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
Express Prep Plate	1	RT
Buffer PB	50	RT
Buffer PW	200	RT
Buffer PE	20	RT
Tape Pad (Sterile)	1	RT
Elution Microtubes (Racked)	96	RT
Caps for Elution Microtubes	96	RT
Hand Book	1	–

9. Specifications

Capacity of a well	800µl
Binding capacity of membrane (ds DNA)	10µg
Recovery of DNA	90-95%
Recovered DNA fragment	(100 bp - 10 kb)
Minimum elution volume	100µl
Total eluate volume	70µl

10. Volumes for a well

Buffer PB	≈ 500µl
Buffer PW	1800µl
Buffer PE	100µl

11. Principle

Obtaining highly pure DNA fragments using mdi Express 96 PCR Purification Kit involves: Capturing PCR amplified DNA on Express prep plate, Washing and Elution.

1. Capturing PCR amplified DNA on Express Prep Plate

   In order to facilitate adsorption of DNA fragments from PCR amplified reactions onto the Express prep plate, 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 Express prep plate, 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

12. 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 Express prep plate.

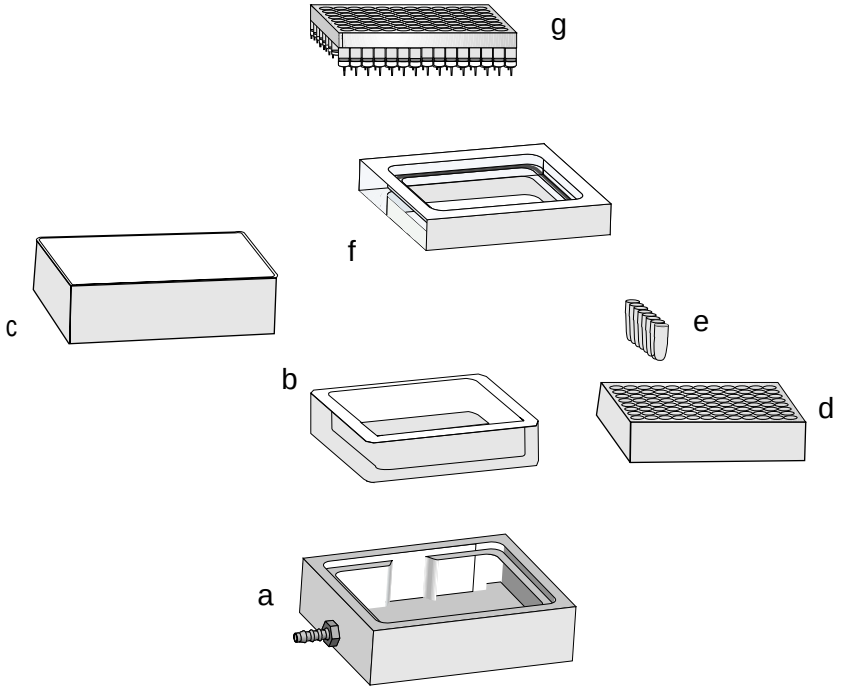
Washing

1. To remove residual wash buffer, apply additional vacuum for 10 minutes.

Elution

1. Elution buffer must be dispensed on to the center of membrane. For maximum elution efficiency, incubation time should be increased by 2-3 minutes.
2. For obtaining highly concentrated DNA, elution should be done in two successive steps with buffer PE in separate elution micro tubes.

13. mdi Vacuum Manifold for 96 Well Purification Kits (VM 96)



mdi VM 96 Manifold Components

a. mdi VM Base

b. Plate Holder

c. Waste Tray

d. Microtube Rack

e. Microtubes

f. mdi VM 96 Top Plate

g. Express Prep Plate

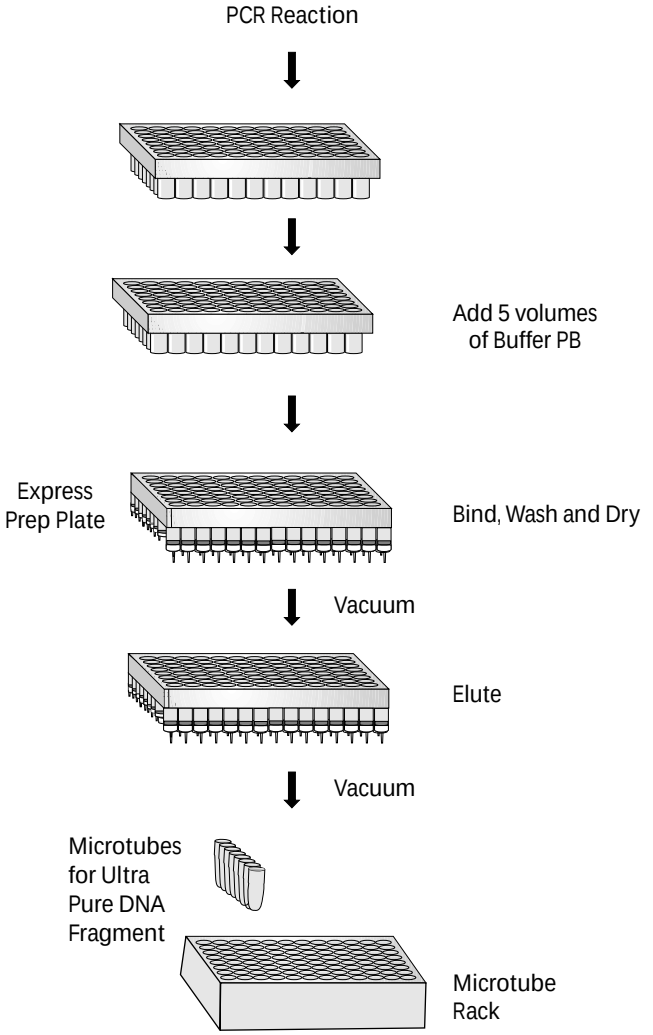
14. Guidelines for mdi (VM 96) Manifolds

1. mdi VM 96 manifold is operated with a house vacuum or Vacuum Pump
2. Always store mdi VM 96 manifolds clean and dry. To clean, simply rinse all components with water and dry with paper towels. Do not use solvents.
3. The components of mdi VM 96 manifold are not resistant to ethanol , methanol ,or other organic solvents. If solvents are spilled on the unit, rinse thoroughly with distilled water. Ensure that no residual Buffer PW should be left in the mdi VM 96 manifold.

Things to be provided by the User

1. mdi VM 96 manifold
2. Multichannel Pipettes
3. Reservoir for multichannel pipettes
4. Vacuum Source
5. Vacuum Regulator

15. mdi Express 96 PCR Purification Procedure



16. Protocol

This protocol is designed for high-throughput PCR Purification using express prep plates on mdi VM 96. The kit accommodates upto 96 parallel purification of up to 10 µg of ultra pure DNA fragment.

Prepare mdi VM 96:

a. Place the Express prep plate in the mdi VM 96 top plate, make sure that the plate is seated securely. Seal unused wells of the Express prep plate with tape.

b. Place the waste tray inside of the mdi VM base.

c. Place mdi VM 96 top plate squarely over base. The express prep plate should now be placed above waste tray. Attach mdi VM 96 to a vacuum source.

d. Regulate (-30 to -150) mm Hg vacuum on empty module using 3 way valve on vacuum regulator on mdi VM 96 before starting the procedure.

17. Procedure

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

2. Load sample mix to the wells of the Express prep plate. Unused wells of the plate should be sealed with tape. Apply vacuum until all samples have passed through.

   The optimal flow rate is approximately 1-2 drops/second which can be regulated by using a 3-way valve or vacuum regulator between the mdi VM 96 and the vacuum source. The flow through is collected in the waste tray.

3. Switch off vacuum. Wash express prep plate by adding 900µl buffer PW to each well and applying vacuum. Repeat wash step once more.

4. After buffer PW has been drawn through all wells, apply maximum vacuum for an additional 10 minutes to dry the membrane.

   Important: Apply maximum vacuum to dry the membrane. Turn off vacuum regulator or leakage valves if they are used. This step is necessary to remove residual wash buffer. Residual buffer may inhibit subsequent down stream applications.

5. Switch off vacuum, and ventilate the mdi VM 96 slowly lift the top plate from the base (do not lift the express prep plate from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the express prep plate with clean absorbent paper. Proceed either to step 6 or 7 as desired.

6. For elution into provided elution microtubes:

   Replace waste tray with the elution microtube rack containing elution microtubes. Place the top plate back on the base, making sure that the express prep plate is seated securely.

7. For elution into a 96-well microplate (not provided):

   Replace waste tray with an empty elution microtube rack (provided with the mdi VM 96). Place a 96-well microplate directly

on the rack. Place the top plate back on the base, making sure that the express prep plate is placed securely.

8. To elute DNA, add 100µl of buffer PE to the center of each well of the express prep plate, let stand for 1 minute and apply maximum vacuum for 5 minutes, switch off vacuum and ventilate mdi VM 96 slowly.

   For increased DNA concentration, an elution volume of 75µl can be used.

18. Trouble Shooting Guide

A. Little or no Yield of DNA

1. Improper dispensing of elution buffer	The elution buffer must be added to the center of the well. Ensure that the entire membrane area is covered by it and no elution buffer lies wasted sticking to the sides of the well.
2. Insufficient incubation of elution buffer in the membrane	Increase incubation time by 2-3 minutes.

B: Low quality DNA

1. Nuclease contamination	Use autoclaved plastic and glassware.

C: DNA does not perform well

Residual wash buffer in eluate	Apply maximum vacuum for 10 minutes to remove residual wash buffer completely.

19. Product Use Limitations

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

20. 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 quality 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.

21. Ordering Information

To order please specify as below:

Type		XX	XX	XX	X	Pack Size	
Type	Code					Pack Size	Code
EPCK	EPCK					1	0001

Example:

EPCK	XX	XX	XX	X	0001

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