MB512 HiPurA® PCR Product Purification Kit

Kit Contents

Product Code	Reagents provided	MB512		
		20 Preps	50 Preps	250 Preps
DS0027	PCR Binding Solution (SPB)	12 ml	30 ml	150 ml
DS0024	Wash Solution Concentrate (HPE)	4 ml	10 ml	50 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	2 ml	5 ml	25 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [inDBCA016 Collection Tube]	20 nos	50 nos	250 nos
DBCA016	Collection Tube (Uncapped) , Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
DBCA017	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

Intended Use

Recommended for isolation of DNA from PCR reactions.

Introduction

The HiPurA® PCR Product Purification Kit provides a simple and rapid method to purify single or double stranded DNA from PCR and other enzymatic reactions. Fragments ranging from 100 bp-10 kb can be purified from primers, nucleotides, polymerases and salts by the use of silica binding in a microspin format that eliminates the need for expensive resins, alcohol precipitation and hazardous organic compounds such as phenol and chloroform. DNA binds specifically to the silica-gel membrane while contaminants such as free nucleotides, primer dimers (upto 50 bp) proteins, $MgCl_2$, mineral oil etc. pass through and are completely removed in wash step. The clean, concentrated DNA is eluted in a small volume of low salt buffer. The purified products are ready to be used in cloning, sequencing and microarray analysis. The PCR product recovery is 80-95% depending on the size of DNA.

HiElute Miniprep Spin Column (Capped) [DBCA03]

HiElute Miniprep Spin Column (Capped) is based on advanced silica binding principle presented in a microspin format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced gel membrane and the speed plus versatility of spin column technology to yield high quantity of DNA. The use of spin column facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. This column eliminates the need for alcohol precipitation, expensive resins and harmful organic compounds such as phenol and chloroform, otherwise employed in traditional DNA isolation techniques. DNA binds specifically to the advanced silica-gel membrane while contaminants pass through.

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

Elution

A single elution with 50 µl of Elution Buffer (ET) will provide sufficient DNA for downstream applications. Purified DNA up to 100 bp-10 kb in length can be purified, and is suitable for direct use in cloning, restriction digestion, sequencing, microarray analysis and southern blotting applications.

Concentration, yield, and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the DNA. Use Elution Buffer (ET) to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/ml of DNA. The $A_{260} - A_{320} / A_{280} - A_{320}$ ratio should be 1.6 - 1.9. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. DNA purified by HiPurA® PCR Product Purification Kit is free of protein and other contaminants that can inhibit enzymatic reactions or any downstream applications.

Concentration of DNA sample (µg/ml) = 50 x A_{260} x dilution factor.

Materials needed but not provided

- 55°C water bath or heating block (if any solution forms precipitate)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96 - 100%)

Storage

Store the HiPurA® PCR Product Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 18 months.

General Preparation Instructions

1. **Thoroughly mix reagents**
   Examine the solutions for any kind of precipitation. If any solution forms a precipitate warm at 55-65°C until the precipitate dissolves completely, allow it to cool to room temperature (15-25°C) before use.

2. Ensure that clean & dry tubes and tips are used for the procedure.

3. **Dilute Wash Solution Concentrate (HPE) (DS0024) as follows:**

Number of Preps	Wash Solution Concentrate (HPE)	Ethanol (96-100%)
20	4 ml	12 ml
50	10 ml	30 ml
250	50 ml	150 ml

Centrifugation

All centrifugation steps are carried out in conventional laboratory centrifuge e.g. Beckman CS-6KR, Heraeus Varifuge 3.0R, or Sigma 6k10 with fixed angle rotor. The tubes provided with the kit are compatible with almost all laboratory centrifuges and rotors. All centrifugation steps are performed at room temperature and are given in g, the correct rpm can be calculated using the formula:

$$RPM = \sqrt{RCF / 1.118 \times 10^{-5} r}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Specimen Collection and Handling

Collect PCR products from PCR reactions. Store PCR products at -20°C. Thaw PCR products before use.

Types of Specimen

Samples: PCR products

Procedure

1. In a 2.0ml capped collection tube, add 5 volumes of PCR Binding Solution (SPB) (DS0027) to 1 volume of the PCR sample and mix well by pipetting. It is not necessary to remove mineral oil.

   For example: Add 500 µl of PCR Binding Solution (SPB) to 100 µl PCR sample (not including oil)

2. Apply the PCR sample / PCR Binding Solution (SPB) mixture to the HiElute Miniprep Spin Column (capped) [DBCA03]. Centrifuge for 1 minute at 12,000 x g (≈13,000 rpm).

3. Discard the flow-through and replace the column in the same collection tube.

4. Add 700 µl diluted Wash Solution (HPE) (DS0024) to the column. Centrifuge for 1 minute at 12,000 x g (≈13,000 rpm) in a tabletop microcentrifuge.

   NOTE: Prepare Wash Solution as indicated in General Preparation Instructions

5. Discard the flow-through and replace the column in the same collection tube.

6. Centrifuge for 1 minute at 12,000 x g (≈13,000 rpm) to remove excess ethanol.

7. Transfer the column to a clean 2.0ml uncapped collection tube, pipette 50 µl of Elution Buffer (ET) (DS0040) to the center of the column and incubate at room temperature (15-25°C) for 1 minute. Centrifuge for 1 minute at 12,000 x g (≈13,000 rpm) in a tabletop microcentrifuge.

   Alternatively, for increased DNA concentration, add 30 µl Elution Buffer to the centre of the column. Incubate at room temperature (15-25°C) for 1 minute and then centrifuge for 1 minute at 12,000 x g (≈13,000 rpm).

8. Transfer the eluate to a fresh capped 2ml collection tube for longer DNA storage.

   The purified PCR amplification product present in the eluate is ready for immediate use. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer (ET) will help to stabilize the DNA at these temperatures.

Warning and Precautions

Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good laboratory practices while handling samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

1. The yield of DNA depends upon the volume of starting material used.

Performance and Evaluation

Each lot of HiMedia's HiPurA® PCR Product Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Recovery
Bacterial PCR product	80-95 %

Reference

1. Sambrook, J., Fritsch, E.F., and Maniatis, T., *Molecular Cloning: A laboratory manual, Cold Spring Harbor Laboratory, second edition (1989).*

Troubleshooting Guide

Sr.No.	Problem	Cause	Solution
1	DNA recovery is low	Improper mixing of Buffer SPB with PCR sample	Ensure that Buffer SPB is mixed properly with PCR sample.
		Elution Buffer was not loaded directly onto the centre of the column	Carefully load the Elution Buffer onto the centre of the column.
2	Poor downstream enzymatic application	Eluate is contaminated with ethanol, which was not completely removed before elution	Be sure to centrifuge at maximum speed in step 6 of the procedure.

Safety Information

The HiPurA® PCR Product Purification Kit is for laboratory use only, not for drug, household or other uses. PCR Binding solution (SPB) contains chaotropic salts, which are irritants. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

Technical assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail to mb@himedialabs.com.

Please refer disclaimer Overleaf.

Symbols

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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