

**MB519**

**HiPurA® 96 PCR Product Purification Kit**

**Kit Contents**

Product Code	Reagents provided	MB519
		1X96 Preps
DS0027	PCR Binding Solution (SPB)	58 ml
DS0024	Wash Solution Concentrate (HPE)	25 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	20 ml
DBPL-96-01	HiPurA® 96-well DNA Plate	1 no
LWB-16-96	HiPurA® 96-well Block (1.6 ml)	2 nos
DVB-96	HiPurA® 96-well V-Block	1 no
PR11	HiPurA® Silicon Pad for Sealing	1 no

**Intended Use**

Recommended for isolation of DNA from Plasmid, tissue, agarose and from PCR reactions

**Introduction**

The HiPurA® 96 PCR Product Purification Kit provides a simple, rapid method for purifying DNA (100 bp-10 kb) from PCR and other enzymatic reactions by the use of silica binding in a 96-well 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<sub>2</sub>, 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 spotting. The PCR product recovery is 80-95% depending on the size of DNA.

**HiPurA® 96-well DNA Plate (DBPL-96-01)**

HiPurA® 96-well DNA Plate is based on the advanced silica binding principle presented in a centrifugation and vacuum format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced silica membrane to yield high quantity of DNA. It facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. It 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. PCR inhibitors such as divalent cations and proteins are completely removed during wash steps, leaving pure nucleic acids to be eluted in the buffer provided with the kit.

**Elution**

A single elution with 100 µl of Elution Buffer (ET) in each well will provide sufficient purified PCR product. PCR product upto 100 bp-10 kb in length can be purified, and is suitable for direct use in cloning, restriction digestion, sequencing, microarray 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® 96 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

- Tabletop Centrifuge with swinging bucket rotor and plate carriers, capable of at least attaining 5,000 rpm
- Vacuum Manifold for 96 well plate
- Vacuum source regulator (pump) (capable of -30 inches Hg pressure)
- Multi-channel pipette with tips
- Ethanol (96-100%)
- Oven preheated at 70°C

### Storage

Store the HiPurA® 96 PCR Product Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 1 year.

### General Preparation Instructions

#### 1. Thoroughly mix reagents

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

#### 2. Dilute Wash Solution Concentrate (HPE) (DS0024) as follows:

Number of Preps	Wash Solution Concentrate (HPE)	Ethanol (96-100%)
1 X 96	25 ml	75 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 96-well accessories 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**

### For Plasmid

Collect overnight culture from sterile flask with the help of micropipette. Store the remaining culture at 2-8°C for short term use.

### For tissues

Collect human/animal cells, tissues, blood sample in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage. Ensure that the tissue is at room temperature before beginning the protocol.

## **Types of Specimen**

Samples: Plasmid cultures, tissues, DNA in agarose gel and PCR products

### Vacuum based Protocol

**NOTE:** Any Vacuum Manifold, which can accommodate 96-well plates, such as the Vacuum Manifold for 96 well plate can be used with the HiPurA® 96-well DNA Plate (DBPL-96-01). A negative pressure of -30 inches Hg is required. We recommend the use of a vacuum regulator to adjust the negative pressure (-30 inches Hg is equivalent to approximately -1000 mbar and -15 psi).

**General Note:** If less than 96 samples are processed at a time, seal the unused wells properly with tape or with the provided HiPurA® Silicon Pad for sealing (PR11).

1. Add 5 volumes of PCR Binding Solution (SPB) (DS0027) to 1 volume of the PCR product and mix well by pipetting. It is not necessary to remove mineral oil.
2. For example, add 500 µl of PCR Binding Solution (SPB) to 100 µl PCR product (not including oil).

**NOTE:** Minimal carryover of oil is expected, but this will not affect the procedure.

3. **Load lysate in HiPurA® 96-well DNA Plate**  
Connect the vacuum manifold to the vacuum source. Remove the manifold top and place a HiPurA® 96-well Block (1.6 ml) (LWB-16-96) into the manifold base to collect the flow-through liquid. Replace the manifold top and place the HiPurA® 96-well DNA Plate (DBPL-96-01) onto the manifold top. Add the PCR sample: PCR Binding Solution (SPB) mixture from step 1 to the corresponding wells of the 96-well DNA Plate. Seal the plate with HiPurA® Silicon Pad (PR11).
4. Turn on the vacuum source and adjust it to -30 inches Hg. Continue to draw vacuum for 10 minutes through the plates or until no liquid remains in any of the wells of the HiPurA® 96-well DNA Plate.
5. Turn off the vacuum source and release the vacuum from inside of the vacuum manifold using the vacuum regulator. Remove the HiPurA® 96-well DNA Plate (DBPL-96-01) from the manifold and temporarily set it aside on a piece of absorbent toweling or plastic wrap.

**NOTE:** Discard the flow-through liquid from HiPurA® 96-well Block (1.6 ml) (LWB-16-96) and reuse it.

6. **Wash**

**(Prepare Wash Solution as indicated in General Preparation Instructions)**

Add 700 µl diluted Wash Solution (HPE) (DS0024) to each well of HiPurA® 96-well DNA Plate. Continue to apply vacuum for 10 minutes through the plates or until no liquid remains in any of the plate wells.

7. Discard the flow-through liquid. Transfer the HiPurA® 96-well DNA Plate in a new HiPurA® 96-well Block (1.6 ml) (LWB-16-96) and apply vacuum at a maximum of –30 inches Hg for 10 minutes to remove the traces of ethanol present in the Wash Solution.

8. Turn off the vacuum source. Remove the HiPurA® 96-well DNA Plate (DBPL-96-01) from the vacuum manifold and vigorously tap the plate approximately 6-8 times on several layers of absorbent toweling.

**NOTE:** Lint-free absorbent toweling is recommended to avoid the release of tiny fibres, which could contaminate the product and interfere with subsequent downstream applications.

9. Incubate HiPurA® 96-well DNA Plate (DBPL-96-01) open without the HiPurA® Silicon Pad for 15 minutes at 70°C in a vacuum oven to evaporate residual ethanol.

**NOTE:** If vacuum oven is not available then connect the vacuum manifold to the oven pre-heated at 70°C through the port provided on the oven.

10. **DNA Elution**

Remove the HiPurA® 96-well Block (1.6 ml) (LWB-16-96) which was used as collection tray from the vacuum manifold base and replace it with a new HiPurA® 96-well V-plate (DVB-96) for elution. Reassemble the manifold top and place the HiPurA® 96-well DNA Plate (DBPL-96-01) on the HiPurA® 96-well V-plate. Add 100 µl of Elution Buffer (ET) to the corresponding wells of HiPurA® 96-well DNA Plate (DBPL-96-01) and incubate at room temperature for 5 minutes. Turn on the vacuum source and allow the vacuum (-30 Hg pressure) to continue for 10 minutes.

11. Turn off the vacuum source and use the vacuum regulator to gradually release the vacuum from the manifold. Disassemble the manifold and remove the HiPurA® 96-well V-plate, which contains eluted purified PCR products.

**Centrifugation based Protocol**

1. 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 product (not including oil).

**NOTE:** Minimal carryover of oil is expected, but this will not affect the procedure.

2. Place the HiPurA® 96-well DNA Plate onto the HiPurA® 96-well Block (1.6 ml) (LWB-16-96). Transfer the PCR sample: PCR Binding Solution (SPB) mixture from step 1 to the corresponding wells of the HiPurA® 96-well DNA Plate. Place the HiPurA® 96-well DNA Plate and HiPurA® 96-well Block into the plate carriers of a swinging bucket rotor. Centrifuge at 5,000 rpm for 5 minutes.

3. **Wash**

**(Prepare Wash Solution as indicated in General Preparation Instructions)**

Remove the HiPurA® 96-well DNA Plate from the centrifuge. Discard the flow-through liquid and reuse the HiPurA® 96-well Block. Add 700 µl of diluted Wash Solution (HPE)

(DS0024) to the HiPurA® 96-well DNA Plate. Reposition the HiPurA® 96-well DNA Plate on the HiPurA® 96-well Block and centrifuge at 5,000 rpm for 5 minutes. Discard the flow-through liquid.

4. Place the HiPurA® 96-well DNA Plate on a new HiPurA® 96-well Block and centrifuge for another 10 minutes at 5,000 rpm to remove the traces of ethanol present in the Wash Solution.

**NOTE:** Only prolonged centrifugation at 5,000 rpm can ensure the complete removal of the traces of the Wash Solution from HiPurA® 96-well DNA Plate membrane.

5. Incubate HiPurA® 96-well DNA Plate (DBPL-96-01) open for 15 minutes at 70°C in an incubator to evaporate residual ethanol.

#### 6. DNA Elution

Remove the HiPurA® 96-well Block (1.6 ml) (LWB-16-96), which was used as collection tray, and replace it with a new HiPurA® 96-well V-plate for elution. Place the HiPurA® 96-well DNA Plate (DBPL-96-01) on it. Add 100 µl of Elution Buffer (ET) (DS0040) to the corresponding wells of HiPurA® 96-well DNA Plate (DBPL-96-01) and incubate at room temperature for 5 minutes. Centrifuge at approximately 56,000 rpm for 5 minutes.

7. Remove the HiPurA® 96-well V-plate (DVB-96), which contains eluted purified PCR products.
8. Alternatively, for increased DNA concentration, add 50 µl Elution Buffer to the centre of the column. Incubate at room temperature (15-25°C) for 5 minutes and then centrifuge at approximately 5,000 rpm.

The PCR amplification product present in the eluate is ready for immediate use. Alternatively, for future use, store at -20°C or lower temperature.

#### 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 type and the volume of starting material used.

#### Performance and Evaluation

Each lot of HiMedia's HiPurA® 96 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 %

## Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	DNA recovery is low	Improper mixing of PCR Binding Solution (SPB) with PCR sample.	Ensure that PCR Binding Solution (SPB) is mixed properly with PCR sample.
		Elution Buffer was not loaded directly into the centre of the well	Carefully load the Elution Buffer into the centre of the well.
		Elution Buffer was left in the well for too long	Allow the Elution Buffer to sit in the well for not more than 5 minutes.
2.	Poor downstream enzymatic application	Eluate is contaminated with ethanol, which was not completely removed before elution	Be sure to apply the vacuum for an additional 10 minutes (step 6 of the vacuum procedure), followed by drying at 70°C, or in case of spin protocol, centrifuge the plate at 5,000 rpm for 10 minutes (step 4) followed by drying at 70°C.
		High salt concentration in eluate	Ensure that the washing step is carried out carefully before the elution, to remove salts.

### Safety Information

The HiPurA® 96 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](mailto:mb@himedialabs.com).

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Please refer disclaimer Overleaf.



Storage temperature



Do not use if package is damaged



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