MB563 HiPurA® PCR Product and Gel Purification Combo Kit

Kit Contents

Product Code	Reagents provided	MB563		
		20 Preps	50 Preps	250 Preps
DS0115	Combo Binding Buffer (CB)	20 ml	50 ml	250 ml
DS0116	Wash Solution Concentrate (WB)	8 ml	20 ml	100 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	2 ml	5 ml	25 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos	50 nos	250 nos
DBCA016	Collection Tube(Uncapped),Polypropylene(2.0ml)	20 nos	50 nos	250 nos
DBCA017	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos	2 X250 nos

Intended Use

Recommended for isolation of DNA from agarose gel and from PCR reactions.

Introduction

The HiPurA® PCR Product and Gel Purification Combo Kit simplifies the purification of nucleic acids from enzymatic reactions viz. PCR as well as from all grades of agarose gels in a single kit. The HiPurA® DNA purification system combines the reversible nucleic acid binding properties of HiElute Miniprep Spin Column with an efficient buffer system, which eliminates contaminants such as proteins.

HiPurA® PCR Product and Gel Purification Combo Kit

The HiPurA® PCR Product and Gel Purification Combo Kit is designed to purify DNA fragments from agarose gels. The simple procedure uses a silica-based spin column to purify DNA fragments (100bp-10kb). It also provides all components to perform a rapid and efficient removal of short primers, dNTPs, enzymes and salts from PCR fragments (100bp-10kb) as well. Chaotropic salt is used to dissolve agarose gel and denature enzymes. DNA fragments in the chaotropic salt are bound to the silica membrane, the contaminants are removed with a Wash Buffer (containing ethanol) and the purified DNA fragments are eluted by a low salt buffer or Molecular Biology Grade Water. Typically, recoveries are upto 80% for gel extraction and 80-95% for PCR product purification. Purified DNA using the HiPurA® kit is free of proteins, dye and agarose, and is ready-to-use for a variety of applications including automated fluorescent DNA sequencing, PCR, *in vitro* transcription, restriction mapping, cloning and labeling.

HiElute Miniprep Spin Column (Capped) [DBCA03]

HiElute Miniprep Spin Column (Capped) is based on the 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.

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

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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. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps, leaving pure nucleic acid to be eluted in the buffer provided with the kit.

Elution

A single elution with 30-50 µl of Elution Buffer (ET) will provide sufficient DNA for downstream applications. Purified DNA up to 100bp-10kb 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 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 and Gel Purification Combo 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

- Microcentrifuge capable of at least 13,000 x g (≈14,000 rpm)
- Water bath or heating block at 55-60°C (For gel extraction protocol or if any solution forms precipitate)
- Ethanol (96-100%)
- 3M Sodium acetate pH 5.2-5.4 (Product code: ML009)
- Nuclease free 1.5 ml microcentrifuge tubes
- Molecular Biology Grade Water (Product code: ML024)

Storage

All HiPurA® PCR Product and Gel Purification Combo Kit components should be stored at room temperature (15°C-25°C) and is stable for upto 18 months without showing any reduction in performance.

General Preparation Instructions

1. Ensure that clean & dry tubes and tips are used for the procedure.
2. Preheat heating block or water bath to 55-60°C (For gel extraction protocol or if any solution forms precipitate).
3. **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 and allow it to cool to room temperature before use.
4. Only up to 400 mg of agarose gel slice can be processed per column.

5. **Dilution of Wash Solution Concentrate (WB) (DS0116) should be freshly prepared before the experiment:**

   Dilute Wash Solution Concentrate (WB) (DS0116) in the ratio 1:4 using ethanol (96-100 %) and mix thoroughly. For example, to 1ml of Wash Solution Concentrate (WB) (DS0116), add 4ml of ethanol (96-100%).

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 for Gel Purification:

NOTE: The yellow color of Combo Binding Buffer (CB) (DS0115) signifies a pH of $\leq$7.5.

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments.

   NOTE: Any type or grade of agarose can be used, but it is strongly recommended that the running buffer (either TAE buffer or TBE buffer) should be fresh. The pH of the buffer may increase by reusing the buffer, which may reduce the final yield.

2. After adequate separation of bands has occurred, excise the DNA bands from the ethidium bromide stained gel with a clean razor blade or scalpel blade using 312 nm UV light and place it in a clean 1.5 ml microcentrifuge tube (not provided).

   NOTE: The size of the gel slice should be minimized by removing extra agarose.

3. Determine the weight of the gel slice and accordingly add three volumes of Combo Binding Buffer (CB) (DS0115) per gel slice volume. Incubate the mixture at 55-60°C for 7 minutes or until the gel has completely melted. Mix the contents of the tube after every 2-3 minutes so that the agarose is completely dissolved.

   NOTE: For example, 100 mg of agarose gel slice requires 300 µl of Combo Binding Buffer (CB) (DS0115). Make sure that the agarose gel slice is solubilized completely.

4. **Load lysate onto HiElute Miniprep Spin Column (Capped)**
   Apply the Gel/Combo Binding Buffer mixture (obtained from the above step) to a HiElute Miniprep Spin Column and centrifuge at 10,000 x g (≈12,000 rpm) for 1 minute at room temperature**.**

NOTE: Approximately, 700 µl of sample mixture can be loaded at a time in the HiElute Miniprep Spin Column (Capped). In case of sample volume larger than 700 µl, discard the flow-through obtained, add rest of the lysate to the column and repeat the spin.

5. Discard the flow-through and place the column back into the same collection tube.

6. Add 300 µl of Combo Binding Buffer (CB) (DS0115) into the column and centrifuge for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature to wash the membrane. Discard the flow through and reuse the collection tube.

7. **Wash**
   (Freshly prepare the Wash Solution Concentrate (WB) (DS0116) as indicated in General Preparation Instructions)
   Place the column into the same collection tube and add 700 µl of diluted Wash Solution. Centrifuge for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature. Discard the flow-through and reuse the collection tube.

   NOTE: If refrigerated, bring Wash Solution (WB) (DS0116) to room temperature, before use.

   OPTIONAL: Repeat Step 8 with another 700 µl of diluted Wash Solution (WB). Discard the flow-through liquid and reuse the collection tube.

   NOTE: The second wash step is for any salt-sensitive downstream applications.

8. Centrifuge the empty column for 2 minutes at maximum speed ≥ 13,000 x g (≥ 14,000 rpm) to dry the column membrane.

   NOTE: This drying step is critical for removal of residual ethanol completely.

9. **DNA Elution**
   Place the column into a new uncapped 2.0 ml collection tube and add 30-50 µl of Elution Buffer (ET) (10mM Tris-Cl, pH 8.5) (DS0040) (depending on the desired concentration of the final product) directly onto the column membrane. Incubate at room temperature for 1 minute. Centrifuge for 1 minute at maximum speed ≥ 13,000 x g (≥ 14,000 rpm) to elute the DNA. Store the eluate into capped 2.0ml collection tube for longer period.

   NOTE: The eluate represents approximately 70% of the bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

Procedure for PCR Product Purification:

1. Add 1 volume of Combo Binding Buffer (CB) (DS0115) to 1 volume of the PCR sample and mix well by pipetting. It is not necessary to remove mineral oil.

   For example: Add 100 µl of Combo Binding Buffer (CB) to 100 µl PCR sample (not including oil)

2. Apply the PCR sample / Combo Binding Buffer (CB) 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 (WB) 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 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 center 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).

   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.

Storage of the eluate with purified DNA: The eluate contains pure DNA. For short-term storage (24-48 hrs) of the DNA, 2-8°C is recommended. 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 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 %
Gel with DNA	80-90 %

Reference

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

Trouble shooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Very less amount of DNA eluted	Combo Binding Buffer (CB) added to the gel slice is too less	Determine the weight of gel slice and accordingly add Combo Binding Buffer (CB) as mentioned in the procedure.

		Incompletely dissolved agarose gel	Allow the gel to completely melt at 55°C-60°C.
		Running Buffer (TAE/TBE) is not fresh	Use fresh (TAE/TBE) Buffer everytime. Avoid over use of (TAE/TBE) buffer as it loses its buffering capacity and its pH increases. This increase in pH interferes with DNA binding to the column membrane. Use of freshly prepared (TAE/TBE) buffer prevents contamination of isolated DNA, and improves recovery.
		Improper mixing of Buffer CB with PCR sample	Ensure that Buffer CB is mixed properly with PCR sample.
		Elution Buffer was not loaded directly onto the center of the column	Carefully load the Elution Buffer onto the center of the column.
		Incomplete elution	Elute the DNA using preheated Elution Buffer (preheated to 70°C) and incubate for 5 minutes before centrifugation.
		Insufficient drying of silica membrane	Centrifuge 5 minutes at maximum speed or incubate column for 2–5 minutes at 70 °C before elution to remove residual ethanol.
		DNA was damaged due to UV light	Reduce UV exposure time to a minimum while excising the band from agarose gel.
2.	Column clogged	Incompletely dissolved agarose gel	Allow the gel to completely dissolve at 55°C-60°C. In case of larger agarose slices ($>$ 0.3 g), it is recommended to slice the gel into smaller fragments to aid the melting.
3.	While loading agarose gel, DNA sample floats out of well	Residual ethanol is not completely removed from column	Centrifuge the empty column at a maximum speed of $\geq$ 13,000 x g ($\geq$ 14,000 rpm) for 2 minutes as mentioned in the protocol.
4.	No DNA eluted	Wash Solution Concentrate (WB) not diluted with ethanol (96-100%)	Refer General Preparation Instructions for correct dilution of Wash Solution Concentrate (WB) with ethanol (96-100%).
		Incorrect amount of Combo Binding Buffer (CB) added	Measure the mass of gel accurately and add 0.3 ml of Combo Binding Buffer (CB) per 0.1 g of gel.
5.	Optical densities do not agree with DNA yield on agarose gel	Trace contaminants eluted from column increase	Ensure that the column is washed as mentioned in the protocol. Alternatively, rely on Agarose gel/ethidium bromide electrophoresis

		A_{260} value	for quantitation.
6.	Appearance of additional bands on agarose gel after gel extraction	Carry-over of residual ethanol	Centrifuge 5 minutes at maximum speed or incubate column for 2–5 minutes at 70 °C before elution to remove residual ethanol.
		Carry-over of chaotropic salts	Ensure that the column is washed as mentioned in the protocol.
7.	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 and Gel Purification Combo Kit is for laboratory use only, not for drug, household or other uses. Combo Binding Buffer (CB) contains chaotropic salts, which are irritants. Avoid direct contact with eyes, skin and clothing. In case of accidental contact, flush affected area with water. Take appropriate laboratory safety measures and wear gloves when handling. 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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