MB539 HiPurA™ Quick Gel Purification Kit

Kit Contents

Product Code	Reagents provided	MB539		
		20 Preps	50 Preps	250 Preps
DS0030	Gel Wash Buffer Concentrate (BIW)	10 ml	25 ml	100 ml
DS0023	Gel Bind Buffer (HG)	40 ml	100 ml	500 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	4 ml	10 ml	30 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	22 nos	55 nos	260 nos
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
PW1139	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos	2X 250 nos

Introduction

The HiPurA Quick Gel Purification Kit simplifies the extraction and purification of nucleic acids from a variety of sources. The HiPurA DNA purification system combines the reversible nucleic acid binding properties of HiElute Miniprep Spin Column (Capped) with an efficient buffer system, which eliminates contaminants such as proteins. The HiPurA Quick Gel Purification Kit simplifies recovery of DNA bands (100 bp-10 kb) from all grades of agarose gels.

HiPurA™ Quick Gel Purification Kit

Gel purification of DNA is a common technique used for isolation of specific DNA fragments from reaction mixtures. However, most methods either fail to completely remove agarose leading to problems in downstream processing, or shearing of DNA resulting in very low yield. This kit uses the HiPurA technology to recover DNA bands of 100 bp-10 kb in length from all grades of agarose gel with yields exceeding 80%. The DNA band of interest is excised from the gel, dissolved in Gel Bind Buffer and applied to HiElute Miniprep Spin Column (Capped). The DNA is eluted with low salt buffer or Molecular Biology Grade Water after a rapid wash step. The product is suitable for downstream applications such as ligation, PCR, sequencing, restriction digestion and various labeling reactions.

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. 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

The yield of DNA depends on the sample type. A single elution with 30-50 µl of Elution

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Buffer (10mM Tris-Cl, pH 8.5) will provide sufficient DNA to carry out multiple amplification reactions. DNA upto 100 bp-10 kb in length can be purified and is suitable for direct use in PCR, ligation, sequencing, restriction digestion, Southern blotting and various labeling reactions.

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 Quick Gel Purification Kit is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

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
- Ethanol (96-100%)
- Nuclease free 1.5 ml microcentrifuge tubes
- Molecular Biology Grade Water (Product code: ML024)

Storage

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

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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.
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. **Gel Wash Buffer (BIW) should be freshly prepared before the experiment:**
   Dilute Gel Wash Buffer Concentrate (BIW) (DS0030) in the ratio 1:4 using ethanol (96-100 %) and mix thoroughly. For example, to 1ml of Gel Wash Buffer Concentrate (BIW), 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.

Procedure

NOTE: The yellow color of Gel Bind Buffer (HG) (DS0023) 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 2.0 ml capped centrifuge tube.

   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 Gel Bind Buffer (HG) (DS0023) 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 Gel Bind Buffer (HG). Make sure that the agarose gel slice is solubilized completely.

4. **Load lysate onto HiElute Miniprep Spin Column**
   Apply the Gel/Gel Binding Buffer mixture (obtained from the above step) to a HiElute Miniprep Spin Column (Capped) 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.

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

7. Add 300 µl of Gel Bind Buffer (HG) 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.

8. **Wash**
   (Freshly prepare the Gel Wash Buffer (BIW) as indicated in General Preparation Instructions)
   Place the column into the same collection tube and add 700 µl of diluted Gel Wash Buffer (BIW) (DS0030). Centrifuge for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature. Discard the flow-through and reuse the collection tube.

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

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

10. 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.

11. **DNA Elution**
    Place the column into a new 2.0 ml uncapped 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.

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

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

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.

Quality Control

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

Trouble shooting Guide:

Sr.No.	Problem	Possible Cause	Solution
1.	Very less amount of DNA eluted	Gel Bind Buffer (HG) added to the gel is too less	Determine the weight of gel slice and accordingly add enough Gel Bind Buffer (HG).
		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.
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 ≥ 13,000 x g (≥ 14,000 rpm) for 2 minutes as mentioned in the protocol.

4.	No DNA eluted	Gel Wash Buffer Concentrate (BIW) not diluted with ethanol (96-100%)	Refer General Preparation Instructions for correct dilution of Gel Wash Buffer (BIW) with ethanol (96-100%).
		Incorrect amount of Gel Bind Buffer (HG) added	Measure the mass of gel accurately and add 0.3 ml of Gel Bind Buffer (HG) per 0.1 g of gel.
5.	Optical densities do not agree with DNA yield on agarose gel	Trace contaminants eluted from column increase A_{260} value	Ensure that the column is washed as mentioned in the protocol. Alternatively, rely on Agarose gel/ethidium bromide electrophoresis for quantitation.

Safety Information

The HiPurA Quick Gel Purification Kit is for laboratory use only, not for drug, household or other uses. Gel Bind Buffer (HG) 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 Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

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.

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