MB563PC16 HiPurA® Pre- filled Cartridges for PCR Product and Gel Purification Combo Kit

Kit Contents

Product Code	Reagents provided	MB563PC16
		48 PR
PF16D2	Pre-filled Cartridges for PCR Product and Gel Purification	48 no
LA1118B	Magnetic Rod Tip	12 no
DS1005A	Magnetic Beads	1.5 ml
DS0023	Gel Bind Buffer (HG)	15 ml
DS0027	PCR Binding Solution (SPB)	15 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH8.5]	4.5 ml

Intended Use

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

Introduction

The HiPurA® Pre- filled Cartridges for 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® Pre- filled Cartridges for PCR Product and Gel Purification Combo Kit

The HiPurA® Pre- filled Cartridges for 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.

Elution

The yield of DNA depends on the sample type. A single elution with 70µl of Elution Buffer 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.

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

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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® Pre- filled Cartridges for PCR Product and Gel Purification Combo 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

- Water bath or heating block at 55-60°C
- Insta NX® Mag16 (LA1118)
- Cartridge Holder (LA1118CH)
- Vortex
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product code: MBLA017)

Storage

Store the HiPurA® Pre- filled Cartridges for PCR Product and Gel Purification Combo Kit between 15-25°C except certain components as specified on each label. Under recommended condition kit is stable for 2 years. We advise a certain storage temperature for the reagents listed below:

- **On receipt store Magnetic Beads (DS1005A): At 2-8°C.**

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 (15-25°C) before use.

Specimen Collection and Handling

Collect DNA from agarose gel and PCR product. Store DNA gel at 2-8°C and PCR product at -20°C. Bring the DNA to room temperature (15-25°C) before use.

Types of Specimen

Samples: DNA in agarose gel, PCR product.

Procedure

Pre- processing for Gel Purification

NOTE: The color of the Gel Bind Buffer (HG) (DS0023) is colorless which eventually turns yellow with time. The pH of the Gel Bind Buffer (HG) (DS0023) is ≤7.5. The change in color of the Gel Bind Buffer (HG) (DS0023) will not affect the quality of the product.

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. **This will be your pre- processed sample**.

   NOTE: For example, 100 mg of agarose gel slice requires 300 µl of Gel Bind Buffer (HG) (DS0023). Make sure that the agarose gel slice is solubilized completely.

Pre- processing for PCR Product Purification

1. In a 2.0ml capped collection tube (not provided), 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. **This will be your pre- processed sample**.

   For example: Add 500 µl of PCR Binding Solution (SPB) (DS0027) to 100 µl PCR sample (not including oil)

Set up processing Cartridges as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Select **"MB563M16"** program. Open the door of the Insta NX® Mag16 machine.
3. Place the Pre-filled Cartridges for PCR Product and Gel Purification (PF16D2) into the Cartridge Holder (LA1118CH). Remove the seal from the **Pre- filled Cartridges for PCR Product and Gel Purification** (PF16D2).

   NOTE: Take care while peeling off the seal. Hold the Cartridge firmly by one hand and then peel off the seal by holding it in your other hand without shaking the Cartridge.

4. Add **50µl of Elution Buffer** (**ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th well** of the Pre-filled Cartridges for PCR Product and Gel Purification (PF16D2).

5. Add **the entire pre-processed sample** obtained in the pre- processing steps of Gel Purification and PCR Product Purification in the **1st well** of Pre- filled Cartridges for PCR Product and Gel Purification **(PF16D2).**

6. Add **20µl of Magnetic beads (DS1005A)** in the **1st well** of the Pre- filled Cartridges for PCR Product and Gel Purification (PF16D2). Place the Cartridges along with the cartridge holder on the platform.

7. Place the Magnetic Rod Tip (LA1118B) onto the machine.

   NOTE: After placing the rods ensure that the rods are properly fixed on their place.

8. Close the door and Click on the **RUN** option on the home screen.

9. After the run is complete, remove Cartridge Holder (LA1118CH) & cartridges from the position. Discard the Magnetic Rod Tip (LA1118B). Dispense the eluted DNA from well 6 to a new HiPer® Lock Microcentrifuge Tube, 2.0ml (not provided). The eluate contains pure DNA.

   NOTE: A small amount of magnetic beads may be observed in the final eluate at the bottom of the plate. Avoid transferring these magnetic beads to your PCR reaction mixture.

   OR

   Take out the eluate in new collection tube (not provided) and centrifuge at higher speed for around 1 min to pellet down the traces of Magnetic beads present in the eluate.

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, recommended to be stored at -20°C or lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA.

Warning

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

Limitations

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

Performance and Evaluation

The yield and efficiency of purification is determined by performing Real- Time PCR.

Quality Control

Each lot of HiMedia's Pre- filled Cartridges for PCR Product and Gel Purification Combo Kit is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre- filled Cartridges for PCR Product and Gel Purification Combo Kit is for laboratory use only, not for drug, household or other uses. 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 of 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.

Symbols

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

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