MB508MPF16 HiPurA® Pre- filled Plates for Plasmid DNA Extraction

Kit Contents

Product Code	Reagents provided	MB508MPF16
		96 PR
PF16Z	Pre-filled Plates for Plasmid DNA Extraction	6 nos
LA1118B	Magnetic Rod Tip	12 nos
DS0003	RNase A Solution (20 mg/ml)	2.8 ml
DS1005A	Magnetic Beads	2 ml
DS0020	Resuspension Solution (HP1)	24 ml
DS0021	Lysis Solution (HP2)	24 ml
DS0022	Neutralization Solution (HN3)	34 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	6.5 ml

Intended Use

Recommended for isolation of plasmid DNA from recombinant E.coli.

Introduction

HiPurA® Pre- filled Plates for Plasmid DNA Extraction provides the fastest and easiest way to purify Plasmid DNA for reliable applications in PCR, library screening, sequencing, etc. The DNA purification procedure comprises of three steps viz. adsorption of DNA to the magnetic beads, removal of residual contaminants and elution of pure plasmid DNA**.**

HiPurA® Pre- filled Plates for Plasmid DNA Extraction

This kit carries out efficient extraction of Plasmid DNA. Sample is first lysed under the highly denaturing conditions provided by Lysis Solution to inactivate RNases and to ensure isolation of intact Plasmid DNA.

Elution

The yield of DNA depends on the sample type and the number of cells in the sample. A single elution with 50µl of Elution Solution will provide sufficient DNA to carry out multiple amplification reactions.

Concentration, yield and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the plasmid 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® Pre- filled Plates for Plasmid DNA Extraction 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.

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WHO GMP CERTIFIED
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Storage

HiPurA® Pre- filled Plates for Plasmid DNA Extraction can be stored at room temperature (15-25°C) for up to 2 years without showing any reduction in performance. We advise a certain storage temperature for the reagents listed below:

- **On receipt store RNase A (DS0003): at 2-8°C.**
- **On receipt store Magnetic Beads (DS1005A): at 2-8°C.**

Materials needed but not provided

- RNase- free pipette tips (aerosol barrier recommended)
- Insta NX® Mag16 (LA1118)
- Vortex
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes) (Tabspin® 012- LA1090)
- 55°C water bath or heating block (if any solution forms precipitate)
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)
- Ethanol (96-100%)

General Preparation Instructions

1. Add 25 µl RNase A Solution (20 mg/ml) per 1 ml of Resuspension Solution (HP1). After the addition of RNase A to HP1 Solution, it is stable for 6 months on storage at 2-8°C.

2. **Thoroughly mix reagents**
   Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.

3. Ensure that clean & dry Nuclease-free tubes and tips are used for the procedure.

4. Vortex magnetic beads before use.

RNase A enzyme treatment

RNase A is a type of RNase that is commonly used in research. RNase A (e.g., bovine pancreatic ribonuclease A) is one of the sturdiest enzymes in common laboratory usage. It cleaves 3'end of unpaired C and U residues.

Unit Definition for RNase A

One unit of the enzyme causes an increase in absorbance of 1.0 at 260 nm when yeast RNA is hydrolyzed at 37°C and pH 5.0. Fifty units are approximately equivalent to 1 Kunitz unit.

It is completely free of DNases and proteases. The specific activity is 90 U/mg. The product as supplied is stable at room temperature (15-25°C).

Specimen Handling and Collection

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.

Types of Specimen

Samples: Bacterial cultures

Pre- processing of culture

- **Harvest Cells**
  Use an overnight (14-16 hours old culture) recombinant *E.coli* culture grown in a medium containing appropriate antibiotic. Take the appropriate volume of the culture into a 2.0 ml capped microcentrifuge tube and centrifuge the cells at ≥12,000 x g [≈13,000 rpm] for 1 minute. Discard the supernatant culture medium.

  NOTE: For good plasmid DNA yield, the $O.D_{600}$ of the culture should be around 3.0×10^6 cells/ml. To calculate the optimal volume of culture to be used, divide the cell mass (3) by the $O.D_{600}$ value.

- **Resuspend Cells**
  Resuspend the bacterial pellet in 250 µl of Resuspension Solution (HP1) (DS0020) and mix well by gentle pipetting till no cell clumps are visible.

  NOTE: It is very important that homogenous suspension is obtained as incomplete resuspension results in poor recovery. Ensure that prior to use, the appropriate amount of RNase A Solution is added to Solution HP1.

- **Lyse Cells**
  Add 250 µl of Lysis Solution (HP2) (DS0021) to lyse the cells. Mix thoroughly by gently inverting the tube 4-6 times.

  NOTE: Do not vortex the tubes as it may result in the shearing of genomic DNA, which may contaminate the plasmid DNA. Do not allow this lysis reaction to exceed more than 5 minutes.

- **Neutralize**
  Add 350 µl of Neutralization Solution (HN3) (DS0022) and immediately mix thoroughly by gently inverting the tube 4-6 times.

  NOTE: The solution should become cloudy.

- Centrifuge the sample at approximately 12,000 x g (≈13,000 rpm) for 10 minutes to obtain a compact white pellet.

  NOTE: A compact white pellet will form. If the supernatant is not clear, transfer the supernatant to a fresh tube and spin for an additional minute at 12,000 x g (≈13,000 rpm) to remove the interfering salts/precipitates completely.

- **This will be your pre- processed sample. Continue with step 1 of set up processing.**

Set up processing as follows:

1. Switch on the UV light 10 mins prior to use.
2. Open the door of Insta NX® Mag16 machine.
3. Select **"MB50816"** program.
4. Remove the seal from the Pre-filled Plates for Plasmid DNA Extraction (PF16Z). Place the Pre-filled Plates for Plasmid DNA Extraction (PF16Z).

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

5. Add **50µl of Elution Buffer** (ET) [10mM Tris-Cl, pH8.5] into **6th and 12th column of the Pre-filled Plates for Plasmid DNA purification (PF16Z).**
6. Add **700ul of pre- processed sample, 20ul of Magnetic Beads (DS1005A) in the 1st and 7th column of the Pre-filled Plates for Plasmid DNA Extraction (PF16Z).** Place the Plate on the platform.
7. Place the Magnetic Rod Tip (LA1118B) by sliding onto the machine.

   NOTE: After placing the rods ensure that the rods are properly fixed on their place.
8. Close the door of Insta NX® Mag16 machine.
9. Click on the **RUN** option on the home screen.
10. After the run is complete, remove Pre-filled Plates for Plasmid DNA Extraction (PF16Z) from the position. Discard Magnetic Rod Tip (LA1118B). Dispense the eluted DNA from column 6 and 12 to a new HiPer® Lock Microcentrifuge Tube, 2.0ml (MBLA017) (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 tube. Avoid transferring these magnetic beads to your PCR reaction mixture.

***NOTE: If you process less than 4 samples at a time please order LA1118B- Magnetic Rod Tip for Insta NX® Mag16 (Pack size- LA1118B-4no/ LA1118B-40no).**

Storage of the eluate with purified DNA: The eluate contains pure plasmid 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 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

Each lot of HiMedia's HiPurA® Pre- filled Plates for Plasmid DNA Extraction is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Yield	DNA Purity
DH5α	upto 20 µg	1.6-1.9

Safety Information

The HiPurA® Pre- filled Plates for Plasmid DNA Extraction 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 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.

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)		Contains biological material of animal origin
	Batch code		

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