MB513 HiPurA® Endotoxin free Plasmid DNA Miniprep Purification Kit

Kit Contents

Product Code	Reagents provided	MB513		
		20 Preps	50 Preps	250 Preps
DS0020	Resuspension Solution (HP1)	7 ml	17.5 ml	87.5 ml
DS0021	Lysis Solution (HP2)	7 ml	17.5 ml	87.5 ml
DS0036	Neutral Solution M (NSM)	10 ml	25 ml	125 ml
DS0025	Endotoxin Removal Solution (ERS)	4 ml	10 ml	50 ml
DS0053	Binding Solution Concentrate (HBC)	8 ml	20 ml	100 ml
DS0032	Wash Solution (HPB)	12 ml	30 ml	150 ml
DS0024	Wash Solution Concentrate (HPE)	4 ml	10 ml	50 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	4 ml	10 ml	50 ml
DS0003	RNase A Solution (20 mg/ml)	36 µl	90 µl	450 µl
PW146	Micro Centrifuge Tube-B (1.5 ml)	40 nos	100 nos	2 X 250 nos
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos	50 nos	250 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	2 X 250 nos

Intended Use

Recommended for isolation of DNA from Plasmid.

Introduction

HiPurA® Endotoxin free Plasmid DNA Miniprep Purification Kit provides a fast and easy method for purification of endotoxin free plasmid DNA from *E.coli* host strains. The DNA purification procedure using the miniprep spin columns comprises of three steps viz. adsorption of DNA to the membrane, removal of residual contaminants and elution of pure plasmid DNA. HiMedia's HiElute Miniprep Spin Column (Capped) format allows rapid processing of multiple samples. The columns have a high binding capacity and high quality DNA is obtained. The endotoxin free plasmid DNA obtained is compatible with downstream applications such as transfection, automated sequencing etc.

HiPurA® Endotoxin free Plasmid DNA Miniprep Purification Kit

Endotoxins, also known as lipopolysaccharides, are present on the cell membrane of Gram negative bacteria such as *E. coli*. During the lysis step of plasmid purification, endotoxins are released, which significantly reduce transfection efficiencies in endotoxin sensitive cell lines.

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Endotoxins represent a non-controllable variable in transfection experiment setup, influencing the outcome and reproducibility of results and making them difficult to compare and interpret. In gene therapy research, endotoxins can interfere by causing endotoxic-shock syndrome and activation of the complement cascade.

The HiPurA® Endotoxin free Plasmid DNA Miniprep Purification Kit integrates an efficient endotoxin removal step into plasmid purification procedure. Cells are lysed and endotoxins are selectively precipitated in a specialized buffer. The resultant lysate is then applied onto the silica column for binding of the DNA molecules in the presence of high salt concentration. The adsorbed DNA is washed to remove contaminants, and the pure plasmid DNA is eluted in Elution Buffer. The purified plasmid DNA can be used for immediate use in all molecular biology procedures such as transfection, automated sequencing and other downstream applications. The kit utilizes an advanced silica-based membrane technology in the form of a convenient spin column, which helps to recover upto 20 µg of high- or low-copy plasmid DNA from 1-5 ml of *E. coli* culture per isolation procedure.

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 silica 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. Plasmid DNA upto 20-30 kb in length can be purified for further downstream applications.

Elution

The yield of plasmid DNA depends on the copy number of the plasmid and the number of cells in the sample. Elution with 50 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 50 µl will increase the final DNA concentration, but will reduce the overall DNA yield.

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® Endotoxin free Plasmid DNA Miniprep 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

- Ethanol (96-100%)
- Water-bath or heating block at 65°C
- Nuclease free microcentrifuge tubes

- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Molecular Biology Grade Water (Product code: ML024)
- 55°C water bath or heating block (if any solution forms precipitate)
- -20°C Freezer

Storage

Store the HiPurA® Endotoxin free Plasmid DNA Miniprep 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. Add 5 µl RNase A Solution (20 mg/ml) (DS0003) per 1ml of Resuspension Solution (HP1). After the addition of RNase A Solution to HP1 Solution, it is stable for 6 months on storage at 2-8°C.

2. Prechill the Neutral Solution M (NSM) before use.

3. **Thoroughly mix reagents**
   Examine the solutions for any kind of precipitation. If any solution (except RNase A) forms a precipitate, warm at 55-65°C until the precipitate dissolves completely and allow it to cool to room temperature before use.

4. Ensure that clean & dry tubes and tips are used for the procedure.

5. **Dilute Binding Solution Concentrate (HBC) (DS0053) with Molecular Biology Grade Water (ML024) as follows:**

Number of Preps	Binding Solution Concentrate (HBC)	Molecular Biology Grade Water
20	8 ml	8 ml
50	20 ml	20 ml
250	100 ml	100 ml

6. **Dilute Wash Solution Concentrate (HPE) (DS0024) as follows:**

Number of Preps	Wash Solution Concentrate (HPE)	Ethanol (96-100%)
20	4 ml	12 ml
50	10 ml	30 ml
250	50 ml	150 ml

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).

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

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

Procedure

1. **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 2.0ml 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.

2. **Resuspend Cells**
   Resuspend the bacterial pellet in 250 µl of Resuspension Solution (HP1) (DS0020) and mix well by gentle vortexing 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 is added to Solution HP1.

3. **Lyse Cells**
   Add 250 µl of Lysis Solution (HP2) (DS0021) to lyse the cells. Mix thoroughly by gently inverting the tube 7-10 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.

4. **Neutralize**
   Add 350 µl of chilled Neutral Solution M (NSM) (DS0036) and immediately mix thoroughly by gently inverting the tube 4-6 times.

   NOTE: The solution should become cloudy and the precipitation should be homogeneous.

5. 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.

6. Transfer the supernatant to a new Microcentrifuge Tube-B (1.5 ml) (PW146) without disturbing the white pellet.

7. Add 0.1 volumes of Endotoxin Removal Solution (ERS) (DS0025) to the lysate. Vortex vigorously at maximum speed until the solution becomes homogeneous. Solution becomes turbid and pink/red in color on addition of ERS Solution. Incubate at -20°C for 5 minutes followed by heating at 65°C for 10 minutes. Centrifuge the tubes at 13,000 x g (≈14,000 rpm) for 5 minutes.

8. Transfer the top aqueous phase to another new Microcentrifuge Tube-B (1.5 ml) (PW146), while avoiding the red viscous phase at the bottom, as this contains concentrated endotoxin.

9. Repeat the extraction procedure one more time as mentioned in step 7. Transfer the colourless upper phase (lysate) to a new 2.0 ml collection tube (not provided) and centrifuge at 13,000 x g (≈14,000 rpm) for 5 minutes. Check for a small red pellet, if present, at the bottom of the tube. Carefully transfer the lysate supernatant to a new 2.0 ml collection tube (not provided), without disturbing the red pellet.

   NOTE: It is critical to obtain the lysate free of any residual Endotoxin Removal Solution.

10. **Bind**
    (Prepare Binding Solution (HBC) (DS0053) as indicated in General Preparation Instructions)
    Add 600 µl of diluted Binding Solution (HBC) to the lysate and mix well by vortexing.

11. **Load lysate onto HiElute Miniprep Spin Column (Capped) [DBCA03]**
    Load the sample onto HiElute Miniprep Spin Column (Capped) and centrifuge at 12,000 x g (≈ 13,000 rpm) for 1 minute. Discard the flow-through liquid and reuse the same collection tube.

12. **First Wash**
    Wash the column by adding 500 µl of Wash Solution (HPB) (DS0032) and centrifuge at 12,000 x g (≈ 13,000 rpm) for 1 minute. Discard the flow-through liquid and reuse the same collection tube.

13. **Second Wash**
    (Prepare Wash Solution (HPE) as indicated in General Preparation Instructions)
    Wash the column by adding 700 µl of diluted Wash Solution (HPE) (DS0024) and centrifuge at 12,000 x g (≈ 13,000 rpm) for 1 minute.

14. Discard the flow-through liquid and centrifuge the tube with the column for an additional 1 minute to remove the traces of Wash Solution.

15. **DNA Elution**
    Transfer the column to a clean uncapped 2.0ml collection tube and add 50 µl of the Elution Buffer (ET) (DS0040) and allow it to stand for 1 minute at room temperature followed by centrifugation for 1 minute at 12,000 x g (≈ 13,000 rpm).

    NOTE: The Elution Buffer is to be added to the centre of the spin column for better recovery. To increase the yield, the elution step can be repeated with another 50 µl of Elution Buffer (ET). However, this will reduce the concentration of the DNA purified.

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

<u>Storage of the eluate with purified DNA:</u> 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 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® Endotoxin free Plasmid DNA Miniprep Purification Kit 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

Troubleshooting Guide

Sr. No.	Problem	Possible Cause	Solution
1.	Poor or low plasmid DNA recovery	Plasmid replication is poor	Ensure that the cells are grown in an appropriate medium under optimized conditions.
		Antibiotic activity is insufficient	Use a fresh antibiotic solution for growth of overnight cultures. Most antibiotic solutions are heat sensitive and degrade during long-term storage at 2-8°C.
		Residual supernatant from cell media	Remove the supernatant after the initial centrifugation; the remaining supernatant can be removed by an additional centrifugation.
		Alkaline lysis is prolonged	The lysis time should be reduced to 3 minutes or until the suspended cells form a clear, viscous solution.
		Precipitation of cell debris is incomplete	The initial volume of cell culture should be reduced or the lysis time can be increased while monitoring the lysis visually.
2.	A_{260}/A_{280} ratio is high or low	Incomplete purification due to overloading of column	The initial volume of the culture should be reduced.
		Background reading is high due to silica fines	Centrifuge the DNA sample at maximum speed for 1 minute, use supernatant to repeat the absorbance readings.

		Wash Solution is diluted with ethanol containing impurities	Check the absorbance of ethanol between 250 nm and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the spin column after washing and contribute to the absorbance in the final product.
		RNA interference, RNase A treatment is insufficient	Ensure that RNase A Solution was added to the Resuspension Solution (HP1) prior to first use.
		Plasmid DNA is contaminated with chromosomal DNA	Do not use cultures that have grown for more than 24 hours or if cells are in the death phase. Do not vortex or vigorously shake the cells during the lysis reaction or neutralization procedure.
3.	Additional band seen ahead of supercoiled plasmid during gel electrophoresis	A portion of the plasmid DNA is permanently denatured	Do not allow the lysis reaction to exceed 5 minutes. **NOTE:** The nicked or covalently open double-stranded plasmid DNA runs slower than the supercoiled DNA during electrophoresis.
4.	Poor performance in downstream enzymatic applications	Purification is incomplete	Salts in one or more of the solutions may have precipitated. 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.
		DNA concentration is too low	Precipitate the DNA with ethanol and then resuspend the DNA in a smaller volume of Elution Buffer. OR Elution of silica-bound DNA can be performed with lesser volumes of Elution Buffer. **NOTE:** By using lesser volume of the Elution Buffer the overall recovery may reduce.
		DNA eluate contains salts	Precipitate the DNA using ethanol. Dry the pellet. Redissolve in water or Elution Buffer.
		The column contains residual ethanol from the diluted Wash Solution	The residual Wash Solution can be removed by re-centrifuging the column at maximum speed for 1 minute (as mentioned in the protocol).

Safety Information

The HiPurA® Endotoxin free Plasmid DNA Miniprep Purification Kit is for laboratory use only, not for drug, household or other uses. The Neutral Solution M (NSM) 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.

Storage temperature

Do not use if package is damaged

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