MB515 HiPurA® Endotoxin free Plasmid DNA Maxiprep Purification Kit

Kit Contents

Product Code	Reagents provided	MB515	
		10 Preps	25 Preps
DS0020	Resuspension Solution (HP1)	130 ml	325 ml
DS0021	Lysis Solution (HP2)	130 ml	325 ml
DS0036	Neutral Solution M (NSM)	130 ml	325 ml
DS0025	Endotoxin Removal Solution (ERS)	50 ml	125 ml
DS0035	Binding Solution (HB)	160 ml	400 ml
DS0034	Wash Solution M (WSM)	160 ml	400 ml
DS0024	Wash Solution Concentrate (HPE)	40 ml	100 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	50 ml	125 ml
DS0003	RNase A Solution (20 mg/ml)	650 µl	1.625 ml
DBCC01	HiPure Maxiprep Column (in PW143 Collection Tube)	10 nos	25 nos
DSYC01	HiPure Maxiprep Syringe Filter	10 nos	25 nos
PW143	Collection Tubes (50 ml conical)	50 nos	125 nos

Intended Use

Recommended for isolation of DNA from Plasmid

Introduction

HiPurA® Endotoxin free Plasmid DNA Maxiprep Purification Kit provides a fast and easy method for purification of endotoxin free plasmid DNA. The DNA purification procedure using the maxiprep 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 HiPure Maxiprep column 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 Maxiprep 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. 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.

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The HiPurA® Endotoxin free Plasmid DNA Maxiprep 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 on to 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 endotoxin free 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 500 µg of high- or low- copy plasmid DNA from upto 500 ml of *E. coli* culture per isolation procedure. The yield depends on plasmid copy number, size of insert, host strain, culture medium and culture volumes. The endotoxin levels are efficiently reduced to less than 0.1 EU/µg.

HiPure Maxiprep Column (DBCC01)

HiPure Maxiprep Column is based on the advanced silica binding principle presented in a spin 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. The eluate obtained 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 1.8-2.0. 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 Maxiprep 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%)
- Nuclease-free pipette tips (with barrier filter)
- Centrifuge with fixed angle rotor for 50 ml tubes capable of ≥ 3,500 x g (5,000 rpm)

- Centrifuge with a swinging bucket rotor for 50 ml tubes capable of ≥ 3,500 x g (5,000 rpm)
- Nuclease-free 50 ml tubes
- Water bath set at 65°C
- Incubator set at 70°C
- Molecular Biology Grade Water (Product code: ML024)
- 3M Sodium acetate pH 5.2-5.4 (Product code: ML009)
- Isopropanol (2-Propanol) (Product Code: MB063)
- 55°C water bath or heating block (if any solution forms precipitate)
- -20°C Freezer

Storage

Store the HiPurA® Endotoxin free Plasmid DNA Maxiprep 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 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, allow it to cool to room temperature before use.

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

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

Number of Preps	Wash Solution Concentrate (HPE)	Ethanol (96-100%)
10	40 ml	120 ml
25	100 ml	300 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 and centrifuge with swinging bucket 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**
   Pellet upto 500 ml of an overnight culture by centrifuging in a fixed angle rotor at 5,000 x g [≈5,000 rpm] for 10 minutes and discard the supernatant. The optimal volume of culture to be used can depend on the strain, the plasmid and density of the culture. **For best results, see the note below.**

   NOTE: For maximum plasmid recovery, begin with a single colony from a freshly streaked plate. Grow in LB medium containing the appropriate antibiotic at 37°C with vigorous shaking (250-300 rpm) overnight. Measure the absorbance at 600 nm. Use a total cell mass of 750, where cell mass equals A_{600} × ml of culture. This is most important when isolating low-copy plasmids and/or using cultures grown in rich medium. To calculate the optimal volume of culture to be used, divide the cell mass (750) by the A_{600} value.

2. **Resuspend Cells**
   Resuspend the bacterial pellet in 12 ml of Resuspension Solution (HP1) (DS0020) and mix well by pipetting up and down or 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 HP1 (as indicated in General Preparation Instructions).

3. **Lyse Cells**
   Add 12 ml of Lysis Solution (HP2) (DS0021) to lyse the cells. Mix thoroughly by gently inverting the tube 6-8 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 12 ml of chilled Neutral Solution M (NSM) (DS0036) and immediately mix thoroughly by gently inverting the tube 4-6 times.

   NOTE: A white aggregate (cell debris, proteins, lipids, SDS and chromosomal DNA) will form.

5. Centrifuge the sample at approximately ≥3500 x g [≥5,000 rpm] in a fixed angle rotor for 10 minutes.

NOTE: After centrifugation, there might be formation of white floating material along with the pellet; both should be avoided as much as possible, while transferring the clear solution.

6. **Prepare filter syringe**
   Prepare a filter syringe by removing the plunger and placing the barrel in a rack to keep the syringe barrel upright. Carefully transfer only the clear solution into the barrel of the filter syringe. The cell lysate will not pass through the filters until the plunger is inserted into the syringe. Allow the lysate to sit for 2 minutes.

7. Hold the filter syringe barrel over a new 50 ml tube and gently insert the plunger to expel all of the lysate into the tube.

8. Add 0.1 volumes of Endotoxin Removal Solution (ERS) (DS0025) to the lysate. Vortex vigorously at maximum speed until the solution becomes homogeneous. The 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 in a fixed angle rotor at ≥3500 x g [≥5,000 rpm] for 10 minutes.

9. Transfer the top aqueous phase to a new 50 ml tube, while avoiding the red viscous phase at the bottom, as this contains concentrated endotoxin.

10. Repeat the extraction procedure one more time as mentioned in steps 8-9. Transfer the colorless upper phase (lysate) to a new 50 ml tube and centrifuge in a fixed angle rotor at ≥3500 x g [≥5,000 rpm] for 10 minutes. Check for a small red pellet, if present, at the bottom of the tube. Carefully transfer the clear lysate supernatant to a new 50 ml tube, without disturbing the red pellet.

    NOTE: This step ensures complete endotoxin removal.

11. **Binding**
    Add 15ml of Binding Solution (HB) (DS0035) to the clear lysate, close the tube and mix thoroughly by gently inverting the tube 3-4 times.

12. **Load lysate to HiPure Maxiprep Column (DBCC01)**
    Load the lysate onto the HiPure Maxiprep Column. Centrifuge in a swinging bucket rotor at ≥3,500 × g (≥5,000 rpm) for 2 minutes. Discard the flow-through and reuse the same collection tube. Repeat the step with the remaining lysate.

13. **First Wash**
    Add 15 ml of Wash Solution M (WSM) (DS0034) to the column and centrifuge in a swinging bucket rotor at ≥3,500 × g (≥5,000 rpm) for 2 minutes. Discard the flow-through and reuse the same collection tube.

14. **Second Wash**
    (Prepare Wash Solution as indicated in General Preparation Instructions)
    Add 15 ml of diluted Wash Solution (HPE) (DS0024) to the column and centrifuge in a swinging bucket rotor at ≥3,500 × g (≥5,000 rpm) for 5 minutes. Discard the flow-through and reuse the same collection tube.

15. Centrifuge the tube with column for an additional 10 minutes to remove traces of Wash Solution at ≥3,500 × g (≥5,000 rpm). Incubate the column at 70°C for 15 minutes.

16. **DNA Elution**
    Transfer the column to a clean new collection tube (provided) and add 1.5 ml of the Elution Buffer (ET) (DS0040) onto the center of the column and centrifuge in a swinging

bucket rotor at ≥3,500 × g (≥5,000 rpm) for 5 minutes. Repeat this elution step one more time in the same tube.

NOTE: Centrifugation at lower speeds will reduce the recovered volume.

17. **DNA concentration (For more concentrated plasmid DNA)**
    Precipitate the plasmid DNA by adding 0.1 volumes of 3.0 M sodium acetate, pH 5.2 and 0.7 volumes of isopropanol to the recovered plasmid DNA. Mix well by inversion and centrifuge at ≥3,500 × g (≥5,000 rpm) at 4°C for 30 minutes. Decant the supernatant, taking care not to disturb the pellet. Rinse the pellet with 1.5 ml of 70 % ethanol and centrifuge at ≥3,500 × g (≥5,000 rpm) at 4°C for 10 minutes. Carefully decant the supernatant and air-dry the pellet until the residual ethanol has evaporated. Resuspend the DNA pellet in 1ml (or desired volume) of Elution Buffer (ET) or Molecular Biology Grade Water (ML024).

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 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 Maxiprep Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Yield	DNA Purity
DH5α	upto 500 µg	1.6-1.9

References

1. Sambrook, J., *et al*. Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989).
2. Birnboim, H.C., and Doly, J. (1979) A rapid alkaline lysis procedure for screening recombinant plasmid DNA. Nuclei Acids Res.7, 1513-1522.
3. Birnboim, H.C., (1983) A rapid alkaline extraction method for the isolation of plasmid DNA. Methods Enzymol.100, 243-255.

Troubleshooting Guide

Sr. No.	Problem	Possible Cause	Solution
1.	Poor or low Plasmid DNA recovery	Number of cells is insufficient	An optimal cell mass of 750 is recommended where cell mass equals A_{600} × ml of culture.

		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 after centrifugation in step 1	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, 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 as mentioned in step 15.

Safety Information

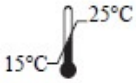
The HiPurA® Endotoxin free Plasmid DNA Maxiprep Purification Kit is for laboratory use only, not for drug, household or other uses. The Neutral Solution M (NSM) contains chaotropic salts, which are irritants. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with 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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