MB514 HiPurA™ Endotoxin free Plasmid DNA Midiprep Purification Kit

Kit Contents

Product Code	Reagents provided	MB514	
		10 Preps	25 Preps
DS0020	Resuspension Solution (HP1)	63 ml	150 ml
DS0021	Lysis Solution (HP2)	63 ml	150 ml
DS0036	Neutral Solution M (NSM)	63 ml	150 ml
DS0025	Endotoxin Removal Solution (ERS)	35 ml	90 ml
DS0035	Binding Solution (HB)	80 ml	190 ml
DS0034	Wash Solution M (WSM)	63 ml	150 ml
DS0024	Wash Solution Concentrate (HPE)	18 ml	40 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	30 ml	70 ml
DS0003	RNase A Solution (20 mg/ml)	315 µl	750 µl
DBCB01	HiPure Midiprep Spin Column (in PW144 Collection Tube)	10 nos	25 nos
DSYB01	HiPure Midiprep Syringe Filter	10 nos	25 nos
PW144	Collection Tube (15 ml conical)	20 nos	50 nos

Introduction

HiPurA Endotoxin free Plasmid DNA Midiprep Purification Kit provides a fast and easy method for purification of total DNA for reliable applications in PCR, library screening and sequencing. The DNA purification procedure using the midiprep 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 Midiprep Spin Column format allows rapid processing of multiple samples. The columns have a high binding capacity and high quality DNA is obtained. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, ligation, PCR and sequencing.

HiPurA™ Endotoxin free Plasmid DNA Midiprep 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.

The HiPurA Endotoxin free Plasmid DNA Midiprep 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 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

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the form of a convenient spin column, which helps to recover upto 25-100 µg of high- or low-copy plasmid DNA from upto 150 ml of *E.coli* culture per isolation procedure. The endotoxin levels are efficiently reduced to less than 0.1 EU/µg.

HiPure Midiprep Spin Column (DBCB01)

HiPure Midiprep Spin Column is based on the advanced silica binding principle presented in a spin column 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 Midiprep 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 15 ml tubes capable of ≥ 3,500 x g (5,000 rpm)
- Nuclease-free 15 ml and 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 Midiprep 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 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 Wash Solution Concentrate (HPE) (DS0024) as follows:**

Number of Preps	Wash Solution Concentrate (HPE)	Ethanol (96-100%)
10	18 ml	54 ml
25	40 ml	120 ml

RNase A enzyme treatment

RNase A is a type of RNase that is commonly used in research to get rid of RNA. 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.

Procedure

1. **Harvest Cells**
   Pellet upto 150 ml of an overnight culture by centrifuging in a fixed angle rotor at 3,500 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 the density of the culture. **For best results, see the NOTE below.**

NOTE: For maximum plasmid recovery, begin with a single isolated colony from a freshly streaked plate. Grow in LB medium containing the appropriate antibiotic at 37°C with vigorous shaking (250-300rpm) overnight (for 16 hours). Measure the absorbance at 600 nm. Use a total cell mass of 250, 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 (250) by the A_{600} value.

2. **Resuspend Cells**
   Resuspend the bacterial pellet in 5 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 Solution (as indicated in General Preparation Instructions).

3. **Lyse Cells**
   Add 5 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. Let the mixture sit for 3-5 minutes until it becomes clear and viscous. Do not allow this lysis reaction to exceed more than 5 minutes.

4. **Neutralize**
   Add 5 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 ≥3,500 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, white floating material as well as pellet, 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. Hold the filter syringe barrel over a new 50 ml tube (not provided), and gently insert the plunger to expel the lysate into the tube.

7. 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.

8. 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.

9. Repeat the extraction procedure one more time as mentioned in steps 7-8. 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.

10. **Bind**
    Add 6 ml of Binding Solution (HB) (DS0035), close the tube and mix thoroughly by gently inverting 3-4 times.

11. **Load onto HiPure Midiprep Spin Column (DBCB01)**
    Transfer the lysate onto the HiPure Midiprep Spin Column placed in 15 ml collection tube (provided). 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. Add rest of the lysate to the column and repeat the spin. Discard the flow-through and reuse the same collection tube.

12. **First Wash**
    Add 5 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.

13. **Second Wash**
    (Prepare Wash Solution as indicated in General Preparation Instructions)
    Add 5 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.

14. Centrifuge the tube with column for an additional 10 minutes in a swinging bucket rotor at ≥3,500 × g (≥5,000 rpm) to remove traces of Wash Solution. Discard any flow-through and the collection tube. Incubate the column at 70°C for 15 minutes.

15. **DNA Elution**
    Transfer the column to a clean new 15 ml collection tube (provided) and add 1 ml of the Elution Buffer (ET) (DS0040) or Molecular Biology Grade Water (ML024) and centrifuge in a swinging bucket rotor at ≥3,500 × g (≥5,000 rpm) for 5 minutes. Repeat the elution step in the same tube.

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

16. **DNA concentration (For more concentrated plasmid DNA)**
    Precipitate the 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. 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 500 µl (or desired volume) of Elution Buffer or Molecular Biology Grade Water.

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.

Quality Control

Each lot of HiMedia's HiPurA Endotoxin free Plasmid DNA Midiprep Purification Kit is tested against predetermined specifications to ensure consistent product quality.

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. Nucleic 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 250 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.
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 14.

Safety Information

The HiPurA Endotoxin free Plasmid DNA Midiprep 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 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 at mb@himedialabs.com.

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