MB508 HiPurA® Plasmid DNA Miniprep Purification Kit

Kit Contents

Product Code	Reagents provided	MB508	
		20 Preps	50 Preps
DS0020	Resuspension Solution (HP1)	7 ml	17.5 ml
DS0021	Lysis Solution (HP2)	7 ml	17.5 ml
DS0022	Neutralization Solution (HN3)	9 ml	22.5 ml
DS0032	Wash Solution (HPB)	12 ml	30 ml
DS0024	Wash Solution Concentrate (HPE)	4 ml	10 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	2 ml	5 ml
DS0003	RNase A Solution (20 mg/ml)	36 µl	90 µl
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos	50 nos
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos
DBCA017	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos

Intended Use

Recommended for isolation of DNA from Plasmid.

Introduction

HiPurA® Plasmid DNA Miniprep Purification Kit provides a fast and easy method for purification of plasmid DNA for reliable applications in PCR, library screening, sequencing, etc. 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 DNA obtained is compatible with downstream applications such as restriction enzyme digestion, ligation, PCR and sequencing.

HiPurA® Plasmid DNA Miniprep Purification Kit

The harvested bacterial culture is lysed and collected by centrifugation after which it is 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 plasmid DNA is eluted in Elution Buffer. The purified plasmid DNA can be used in all molecular biology procedures such as digestion with restriction enzymes, cloning, PCR, transfection, *in vitro* translation, blotting and sequencing. 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.

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

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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. A single 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® 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%)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Molecular Biology Grade Water (Product Code: ML064)
- 55°C water bath or heating block (if any solution forms precipitate)

Storage

Store the HiPurA® Plasmid DNA Miniprep Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 18 months.

General Preparation Instructions

1. Add 5 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 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 (15-25°C) before use.
3. Ensure that clean & dry tubes and tips are used for the procedure.

4. **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 (15-25°C) 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 2.0 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.

2. **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.

3. **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.

4. **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.

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. **Load lysate onto HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Carefully remove the supernatant and transfer onto a HiElute Miniprep Spin Column (Capped) and centrifuge at 12,000 x g (≈13,000 rpm) for a minute. Discard the flow-through liquid.

7. **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 a minute. Discard the flow-through liquid.

8. **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.

9. Discard the flow-through liquid and centrifuge the empty tube with the column for an additional 1 minute at same speed to remove any traces of the Wash Solution.

10. **DNA Elution**
    Transfer the column to a clean 2.0 ml uncapped collection tube and add 50 µl of the Elution Buffer (ET) (DS0040) or Molecular Biology Grade Water (ML064). Allow it to stand for 1 minute at room temperature (15-25°C), followed by centrifugation for 1 minute at 12,000 x g (≈ 13,000 rpm).

    NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer (ET) and then centrifuge. Elution with volumes less than 50 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. Storing DNA in water can cause acid hydrolysis.

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

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® 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

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	Ensure that the cell density is $3X10^6$ cells.
		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 and 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	1. Do not use cultures that have grown for more than 24 hours or if cells are in the death phase. 2. Do not vortex or vigorously shake the cells during the lysis reaction or neutralization procedure.
3.	Additional band seen ahead of supercoiled plasmid DNA 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.
		Incorrect addition of Lysis Solution (HP2)	Ensure that the lysate is handled gently after adding Lysis Solution (HP2) to prevent shearing. If the lysate is too viscous for gentle mixing, reduce the culture volume.
		Incorrect addition of Neutralization Solution (HN3)	Upon addition of Neutralization Solution (HN3), mix immediately but gently.
		Overgrown culture	Do not grow cultures for longer than 14-16 hours. Such cultures contain lysed cells and degraded DNA.
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 (15-25°C) 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.

Safety Information

HiPurA® Plasmid DNA Miniprep Purification Kit is for laboratory use only; not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Avoid contact with skin, and use eye protection. In case of contact, wash with large amount of water. Seek medical attention. 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
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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