MB568A HiPurA® Food Pathogen (Bacteria) DNA Purification Kit

Kit Contents

Product Code	Components	MB568A	
		10PR	25 PR
DS0014	Gram Positive Lysis Solution (GPLA)	3 ml	7.5 ml
DS0015	Lysis Solution (AL)	3 ml	7.5 ml
DS0010	Lysis Solution (C1)	3 ml	7.5 ml
DS0031	Prewash Solution (PWB)	6 ml	15 ml
DS0012	Wash Solution Concentrate (WS)	2 ml	5 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH8.5]	3 ml	7.5 ml
MB086	Proteinase K	5 mg	12.5 mg
DS0003	RNase A Solution (20mg/ml)	250 µl	625 µl
MB098	Lysozyme	140 mg	350 mg
DSCA02	HiShredder (in DBCA016 Collection Tube)	10 nos.	25 nos.
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	10 nos.	25 nos.
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0ml)	10 nos.	25 nos.
PW1139	Collection Tube, Polypropylene (2.0ml)	20 nos.	50 nos.

Intended Use

Recommended for isolation of DNA from Food samples.

Introduction:

HiPurA® Food Pathogen (Bacteria) DNA Purification Kit is developed to provide a fast and easy method for purification of bacterial DNA from variety of food samples (after enrichment according to AOAC guidelines) for reliable applications in PCR. The DNA purification procedure using the miniprep spin column comprises of three steps viz. adsorption of DNA to the membrane, removal of residual contaminants and elution of pure genomic 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 genomic DNA is obtained from various species. The DNA obtained is compatible with downstream applications like PCR.

HiPurA® Food Pathogen (Bacteria) DNA Purification Kit

This kit simplifies isolation of DNA from food samples by the spin-column procedure. The food sample is enriched in an enrichment medium (not provided) as per AOAC guidelines and the cells are harvested by centrifugation. After harvesting, the bacterial (Gram positive) cell wall is degraded by lysozyme and Proteinase K. For Gram negative bacteria, the lysozyme treatment is not required.
Following lysis, the DNA is bound to the silica-gel membrane of the HiElute Miniprep Spin Column (Capped) to yield pure DNA. Two rapid wash steps remove trace amount of salt and protein contaminants resulting in the elution of pure DNA in the Elution Buffer provided with the kit.

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

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

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

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. The purified DNA can be used for further downstream applications.

Elution

The yield of genomic DNA depends on the sample type and the number of cells in the sample. A single elution with 200 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 200 µl will increase the final DNA concentration, but will reduce the overall DNA yield.

Materials needed but not provided

- 55°C water bath or heating block
- 37°C water bath or heating block
- Tabletop Micro centrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96 - 100%)
- Micropipettes and Tips
- Distilled water
- Autoclave
- Lysostaphin (Optional) (For *Staphylococcus* species only)
- Mutanolysin (Optional) (For *Streptococcus* species only)
- Molecular Biology Grade Water (Product code: ML024)
- Incubator Shaker
- **Selective Enrichment Media for sample preparation as per AOAC guideline :**

  For e.g. Buffered Peptone water (M1494I) for Salmonella & E.CO157:H7: Suspend 20.07 g (the equivalent weight of dehydrated medium per liter) in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense in tubes or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Storage

Store the HiPurA® Food Pathogen (Bacteria) DNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended conditions, the kit is stable for 1 year.

General Preparation Instructions

1. Preheat a water bath or heating block to 55°C.
2. Preheat a water bath or heating block to 37°C (for Gram positive bacteria only).
3. **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.
4. Ensure that clean & dry tubes and tips are used for the procedure.

5. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100%)
10	2 ml	8 ml
25	5 ml	20 ml

6. **Reconstitute Proteinase K (MB086)**

   The HiPurA® Food Pathogen DNA Purification Kit (Salmonella) contains Proteinase K. It is completely free of DNase and RNase activity. The specific activity of Proteinase K is 33.5 units/mg dry weight.

   Re-suspend the Proteinase K (MB086) powder in Molecular Biology Grade Water (ML024) to obtain a 20 mg/ml stock solution.

Number of Preps	Proteinase K	Molecular Biology Grade Water
10	2 mg	0.1 ml
25	12.5 mg	0.62 ml

   The product as supplied is stable at room temperature (15-25°C); upon receipt, store at 2-8°C & upon reconstitution store at -20°C.

7. **Prepare Lysozyme Solution (Product Code: MB098) [For Gram positive bacteria only]**

   Prepare a 45mg/ml solution of Lysozyme (approximately 2.115 x 10^6 unit/ml) with the Gram Positive Lysis Solution (GPLA) (DS0014) as the diluent, which is provided in the kit. Lysozyme solution should be freshly prepared prior to use.

   Example: In order to make 1 ml of Lysozyme solution, dissolve 45 mg of lysozyme (provided) in 1 ml of Gram Positive Lysis Solution (GPLA) (DS0014). Pipette the mixture up and down or vortex to dissolve the lysozyme.

   NOTE: Lysozyme dissolves readily by pipetting up and down as opposed to vortexing. Excessive vortexing may cause foaming.

   For each DNA preparation, 200 µl of lysozyme solution is required. Make extra solution to account for pipetting error. The lysozyme solution should be preferably used on the day of preparation. If some Lysozyme stock solution is left, it can be stored at -20°C.

8. **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 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.

Enrichment of pathogens:
In order to ensure sensitive detection of pathogens from different variety of food products by PCR, the pathogens need to be enriched in broth.

Specimen Collection and Handling

For Food
Collect food sample in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage.

Types of Specimen

Samples: Food

Procedure:

A. Gram Negative Bacterial Preparation

1. **Harvesting of cells**
   Pellet 1.5 ml of an overnight enriched sample in provided 2ml capped collection tube by centrifuging for 2 minutes at 12,000-16,000 x g (approximately 13,000-16,000 rpm). Remove the culture medium and discard.

2. **Re-suspend the pellet**
   Re-suspend the pellet thoroughly in 180 µl of Lysis Solution (AL) (DS0015).

3. **Prepare for cell lysis**
   Add 20 µl of the Proteinase K solution (20mg/ml) **(Refer to General Preparation Instructions)** to the sample. Mix and incubate for 5 minutes at 55°C. If residual RNA is not a concern, continue with step 4 of Gram Negative Bacterial Preparation.

   Optional RNase A treatment
   If RNA-free genomic DNA is required, add 20 µl of RNase A Solution (DS0003), mix and incubate for 5 minutes at room temperature (15-25°C), then continue with step 4 of Gram Negative Bacterial Preparation.

4. **Lyse cells**
   Add 200 µl of Lysis Solution (C1) (DS0010), vortex thoroughly (about 15 seconds) and incubate at 55°C for 10 minutes, then continue with step C (Load sample in HiShredder).

   NOTE: A homogeneous mixture is essential for efficient lysis.

B. Gram Positive Bacterial Preparation

1. Prepare Lysozyme Solution using Lysozyme from chicken egg white, which is provided in the kit. Prepare a 45 mg/ml stock solution of lysozyme as described under General Preparation Instructions. 200 µl of Lysozyme Solution is required per isolation procedure. Prepare extra solution to account for pipetting error.

   NOTE: (Optional) For higher yields - If working with *Staphylococcus* species, supplement the Lysozyme Solution with 200 units/ml of lysostaphin. For *Streptococcus* species, supplement the Lysozyme Solution with 250 units/ml of mutanolysin.

2. **Harvest Cells**
   Pellet 1.5 ml of bacterial broth culture in provided 2ml capped collection tube by centrifuging for 2 minutes at 12,000-16,000 x g (≈13,000-16,000 rpm). Remove the culture medium completely and discard.

3. **Resuspend cells**
   Resuspend the pellet thoroughly in 200 µl of lysozyme solution (prepared in step 1b) and incubate for 5 minutes at 37°C.

4. **Lyse cells**
   Add 20µl of the Proteinase K Solution (20 mg/ml) **(Refer to General Preparation Instructions)** to the sample. If residual RNA is not a concern continue with step 5 of Gram Positive Bacterial Preparation.

   Optional RNase A treatment
   If RNA-free genomic DNA is required, add 20 µl of RNase A Solution (DS0003), mix and incubate for 5 minutes at room temperature (15-25°C), then continue with step 5 of Gram Positive Bacterial Preparation.

5. Add 200 µl of Lysis Solution (C1) (DS0010). Vortex thoroughly for few seconds and incubate at 55°C for 10 minutes, then continue with step C (Load sample in HiShredder).

   NOTE: A homogeneous mixture is essential for efficient lysis.

DNA ISOLATION PROCEDURE

This is a continuation of the procedure from the lysates prepared in steps 1a-4a and 1b-5b.

C. Load sample in HiShredder (DSCA02)
Add the entire sample to the HiShredder placed in a 2.0 ml collection tube (uncapped) and spin for 1 minute at a maximum speed (≈13,000 rpm). Transfer the flow-through fraction to a 2.0 ml collection tube (not provided) without disturbing the cell debris pellet.

D. Prepare for binding
Add 200 µl of ethanol (95-100%) to the lysate and mix thoroughly by vortexing for few seconds.

NOTE: A homogenous mixture is essential. A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the HiElute Miniprep Spin Column. This precipitate does not interfere with the DNA isolation procedure or with any subsequent application. Do not use alcohols other than ethanol because this may result in reduced yields.

E. Load lysate onto HiElute Miniprep Spin Column (Capped) [DBCA03]
Transfer the lysate obtained from step D onto HiElute Miniprep Spin Column (Capped) provided. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and place the spin column in same 2.0 ml collection tube.

NOTE: Use a wide bore pipette tip to reduce shearing of the DNA while transferring contents onto the column. It is essential to apply all of the precipitate to the column. If the solution has not completely passed through the membrane, spin at a higher speed until all the solution has passed through. Centrifugation at full speed will not affect the yield or purity of the DNA.

F. Prewash
Add 500 µl of Prewash Solution to the column and centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

G. Wash

(Prepare diluted Wash Solution (WS) (DS0012) as indicated in General Preparation Instructions)

Add 500 µl of diluted Wash Solution (WS) to the column and centrifuge for 3 minutes at maximum speed 12,000-16,000 x g (≈13,000-16,000 rpm). Discard the flow through and spin again at same speed for the additional 1 minute to dry the column. The column must be free of ethanol before eluting the DNA.

H. DNA Elution

Transfer the HiElute Miniprep Spin Column (Capped) to fresh uncapped collection tube. Pipette 200 µl of the Elution Buffer (ET) (DS0040) directly into the column without spilling to the sides. Incubate for 1 minute at room temperature. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute to elute the DNA.

NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Buffer (ET), then centrifuge. Elution with volumes less than 200 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.

I. Transfer the eluate to a fresh capped 2ml collection tube for longer storage. This eluate can be used as template for PCR.

Storage of the eluate with purified DNA: The eluate contains pure genomic 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® Food Pathogen (Bacteria) DNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Purity
Fruit juice	1.6-1.9

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Lysozyme is difficult to dissolve	Solution is inadequately mixed	Pipette repeatedly to dissolve the lysozyme as opposed to vortexing; as vortexing will cause foaming and reduce lysozyme solubility. Lysozyme may not

			dissolve readily; complete dissolution is not needed prior to use as it will dissolve during the 37°C incubation.
2.	HiElute Miniprep Spin Capped Column is clogged	Sample volume is large	Use smaller quantity of sample; to salvage the current preparation, clogging can be alleviated by increasing the 'g' force or spinning for longer time until the lysate passes through the spin column.
3.	Lysate appears to be gelatinous prior to loading onto the column	Sample volume is large	Use fewer cells (≤ $1x10^{10}$ cells/ml). The incubation time and or the amount of Proteinase K solution can be increased. Eg: The incubation time and the amount of enzyme can be doubled.
4.	Poor / Lower yield of genomic DNA	Sample is old	Yield of genomic DNA varies from different species and strains of bacteria. It is necessary to use cells before they reach their maximum density or they become confluent.
		Incomplete lysis of cells	The incubation time and or the amount of Proteinase K solution can be increased. eg: The incubation time and the amount of enzyme can be doubled.
		Lysate/Ethanol mixture is not homogenous	Vortex the tubes for atleast 5-10 seconds in order to obtain a homogenous solution before applying it to the column.
		DNA elution is incomplete. Eluate contains residual ethanol from the wash	• DNA yield can be improved by incubating the Elution Buffer for 5 minutes at room temperature (15-25°C) after it is added to the column. • Ethanol from the final wash should be eliminated completely before eluting DNA. Spin the tubes for longer time to dry the column completely.
		Wash Solution Concentrate was not diluted before use	Check that the Wash Solution Concentrate is properly diluted with ethanol as per instructions.
		Use of water instead of Elution Buffer for elution of DNA.	Elution Buffer is recommended for optimal yields and storage of the genomic DNA. If water is used instead of the Elution Buffer the pH should be at least 7.0 to avoid acidic conditions, which may cause acid hydrolysis of DNA when stored for long periods of time. (**NOTE:** Only DNase/RNase and Protease free water should be used for eluting DNA)
5.	Downstream	Traces of ethanol	After the washing steps, the eluate

	applications are inhibited	present in the final genomic DNA preparation	should not come in contact with the column. Spin the column for 1 minute at maximum speed (12,000-16,000 x g) if necessary, after emptying the collection tube.
		Salt is carried over in the final genomic DNA preparation	The HiElute Miniprep Spin Capped Column should be transferred to a new 2.0 ml collection tube before adding the wash solution.

Safety Information

HiPurA® Food Pathogen (Bacteria) DNA 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.

Storage temperature

Do not use if package is damaged

HiMedia Laboratories Private Limited,
Reg. Off: Plot No. C-40, Road No. 21Y,
MIDC, Wagle Industrial Area, Thane,
(West) 400604, Maharashtra, INDIA.
Web: www.himedialabs.com

12/2024

HiMedia Laboratories Pvt. Ltd. Reg.office : Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797 Tel: 00-91-22-6147 1919, 6903 4800 Email: techhelp@himedialabs.com Website: www.himedialabs.com