MB548 HiPurA® 96 Bacterial Genomic DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB548
		1 X 96 Preps
DS0014	Gram Positive Lysis Solution (GPLA)	24 ml
DS0015	Lysis Solution (AL)	24 ml
DS0010	Lysis Solution (C1)	24 ml
DS0031	Prewash Solution (PWB)	57.6 ml
DS0012	Wash Solution Concentrate (WS)	24 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	19.2 ml
MB086	Proteinase K	48 mg
DS0003	RNase A Solution (20 mg/ml)	2.4 ml
MB098	Lysozyme	1.44 g
LWB-16-96	HiPurA® 96-well Block (1.6 ml)	2 no.
DBPL-96-01	HiPurA® 96-well DNA Plate	1 no.
PR11	HiPurA® Silicon Pad for sealing	1 no.
DVB-96	HiPurA® 96-well V-plate	1 no.
LWB-96	HiPurA® 96-well Block (2.2 ml)	1 no.

Intended Use

Recommended for isolation of DNA from bacterial cultures

Introduction

The HiPurA® 96 Bacterial Genomic DNA Purification Kit provides a fast and easy method for purification of total DNA for reliable applications in PCR, Southern blotting technique etc. HiMedia's 96-well format allows rapid processing of multiple samples. The DNA obtained is compatible with down stream applications such as restriction enzyme digestion, ligation, PCR and sequencing.

HiPurA® 96 Bacterial Genomic DNA Purification Kit

This kit simplifies isolation of DNA from bacteria (Gram positive and Gram negative) by a simple procedure. Bacterial cells are grown in a medium till they reach log phase and 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 HiPurA® 96-well DNA Plate (DBPL-96-01) to yield approximately upto 20 µg of 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.

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

HiPurA® 96-well DNA Plate (DBPL-96-01)

HiPurA® 96-well DNA Plate is based on the advanced silica binding principle presented in a centrifugation and vacuum format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced silica membrane to yield high quantity of DNA.

It facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. It 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 during wash steps, leaving pure nucleic acids to be eluted in the buffer provided with the kit. Bacterial genomic DNA upto 20-30 kb in length can be purified for further downstream applications.

Elution

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. The eluted DNA ranges in size up to 20-30 kb, and is suitable for direct use in PCR, restriction digestion, and southern blotting applications.

Concentration, yield and purity

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic 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® 96 Bacterial Genomic DNA 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

- 37°C water bath or heating block
- 70°C water bath or heating block
- Tabletop Centrifuge with swinging bucket rotor and plate carriers, capable of atleast attaining 2,250 x g (≈3,700 rpm)
- Ethanol (96-100%)
- Lysostaphin (Optional) (For *Staphylococcus* species only)
- Mutanolysin (Optional) (For *Streptococcus* species only)
- Molecular Biology Grade Water (Product code: ML064)

Storage

HiPurA® 96 Bacterial Genomic DNA Purification Kit can be stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance. The Proteinase K solution (after reconstitution) can be stored for several days at 2-8°C. For long-term storage, the unused portion of the solution may be stored in aliquots at -20°C until needed. The product as supplied is stable at room temperature (15-25°C).

General Preparation Instructions

1. Preset a water bath or heating block to 70°C (For Gram positive, Gram negative bacteria).

2. Preheat a water bath or heating block to 37°C (For use with 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. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100%)
1X96 PR	24 ml	96 ml

   NOTE: ***User should carry out dilution of Wash Solution Concentrate (WS) in a separate container, as it is not provided with this kit.***

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

   The HiPurA® 96 Bacterial Genomic DNA Purification Kit contains Proteinase K. Intensive research has shown that it is the optimal enzyme for use with the Lysis Solution provided in the kit. It is completely free of DNase and RNase activity. Proteinase K is the enzyme of choice for use with an SDS containing Lysis Solution. The specific activity of Proteinase K is 33.5-units/mg dry weight.

   Resuspend 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
1X96 PR	48 mg	2.4 ml

   The product as supplied is stable at room temperature (15-25°C); upon reconstitution store at -20°C as mentioned in storage instructions.

   NOTE: The Proteinase K solution must be added directly to each sample preparation every time. Do not combine the Proteinase K and Lysis Solution for storage.

6. **Prepare Lysozyme Solution (Product Code: MB098) For Gram positive bacteria only**
   Prepare a 45 mg/ml solution of Lysozyme (MB098) (approximately 2.115 x 10^6 unit/ml) with the Gram Positive Lysis Solution (GPLA) 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). 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.

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 swinging bucket rotor for 96-well plate. The 96-well accessories 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.

General Note:

1. If less than 96 samples are processed at a time, seal the unused wells properly with the provided HiPurA® Silicon Pad for sealing (PR11).

2. The HiPurA® Silicon Pad for sealing (PR11) used in the protocol should not be discarded. It can be reused after wiping with ethanol and washing properly.

3. Vacuum as well as centrifugation protocol can be performed. For a vacuum protocol, negative pressure of - 30 mm Hg is required to achieve optimum results.

Specimen Collection and Handling

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: Bacterial culture

Procedure:

Vacuum based protocol

A. Gram Negative Bacterial Preparation

1. **Harvesting of cells**

   Pellet 1 ml of overnight bacterial broth culture in the HiPurA® 96-well Block (2.2 ml) (LWB-96) by centrifugation at 2250 x g (3,700 rpm) for 10 minutes at room temperature (15-25°C) in a centrifuge with a rotor and carriers/adaptors for 96-well microplates. The block should be covered with HiPurA® Silicon Pad for sealing (PR11) during centrifugation. Remove the medium by gently inverting the block onto a waste container.

   NOTE: Tap the inverted block gently onto an absorbent paper towel stack to drain out the remaining droplets of liquid. Ensure that the pellet is firm and does not get disturbed during tapping.

   NOTE: If bacteria are grown in rich media such as terrific broth, it is necessary to reduce the volume of the starting material of the overnight broth culture to 0.5 ml in order to avoid overloading of the HiPurA® 96-well DNA Plate (DBPL-96-01)

2. **Resuspend cells**
   Resuspend each bacterial pellet in 180 µl of Lysis Solution (AL) (DS0015). Seal the HiPurA® 96-well Block (2.2 ml) (LWB-96) using the provided HiPurA® Silicon Pad for sealing (PR11). Mix well by gentle vortexing till no cell clumps are visible. Remove the silicon pad from the top of the HiPurA® 96-well Block (2.2 ml) (LWB-96)

   NOTE: It is very important that homogenous suspension is obtained as incomplete resuspension will result in poor recovery.

3. **Prepare for cell lysis**
   Add 20 µl of the Proteinase K solution (20 mg/ml) to each well of 96-well plate. Mix and incubate for 30 minutes at 70°C. If residual RNA is not a concern, continue with step 4.

   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.

4. **Lyse cells**
   Add 200 µl of Lysis Solution (C1) (DS0010), to each well of HiPurA® 96-well Block (2.2 ml) (LWB-96) to lyse the cells. Seal the HiPurA® 96-well Block (2.2 ml) (LWB-96) using the HiPurA® Silicon Pad provided with the kit (PR11). Vortex thoroughly (about 15 seconds) and incubate at 70°C for 10 minutes. Continue with step 5 (Prepare for binding).

   NOTE: A homogeneous mixture is essential for efficient lysis.

B. <u>Gram Positive Bacterial Preparation</u>

Prepare Lysozyme Solution using Lysozyme from chicken egg white, which is provided in the kit (MB098). Prepare a 45 mg/ml stock solution of lysozyme as described under General Preparation Instructions. 200 µl of Lysozyme Solution is required per well. 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.

1. **Harvesting of cells**
   Pellet 1 ml of overnight bacterial broth culture in the HiPurA® 96-well Block (2.2 ml) (LWB-96) by centrifugation at 2250 x g (3,700 rpm) for 10 minutes at room temperature (15-25°C) in a centrifuge with a rotor and carriers/adaptors for 96-well microplates. The block should be covered with HiPurA® Silicon Pad for sealing (PR11) during centrifugation. Remove the medium by gently inverting the block onto a waste container.

   NOTE: Tap the inverted block gently onto an absorbent paper towel stack to drain out the remaining droplets of liquid. Ensure that the pellet is firm and does not get disturbed during the tapping.

   NOTE: If bacteria are grown in rich media such as Terrific broth, it is necessary to reduce the volume of the starting material of the overnight broth culture to 0.5 ml in order to avoid overloading of the HiPurA® 96-well DNA Plate (DBPL-96-01).

2. **Resuspend cells**
   Resuspend the pellet thoroughly in 200 µl of lysozyme solution (prepared in step 1 of Gram Positive Bacterial Preparation) and incubate for 30 minutes at 37°C.

3. **Prepare for cell lysis**
   Add 20 µl of the Proteinase K solution (20 mg/ml) to each well of 96-well plate. If residual RNA is not a concern, continue with step 4.

   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.

4. **Lyse cells**
   Add 200 µl of Lysis Solution (C1) (DS0010) to each well of HiPurA® 96-well Block (2.2 ml) (LWB-96) to lyse the cells. Seal the HiPurA® 96-well Block (2.2 ml) (LWB-96) using the HiPurA® Silicon Pad provided with the kit (PR11). Vortex thoroughly (about 15 seconds) and incubate at 70°C for 10 minutes. Continue with step 5 (Prepare for binding).

   NOTE: A homogeneous mixture is essential for efficient lysis.

DNA ISOLATION FROM GRAM POSITIVE, GRAM NEGATIVE BACTERIA

This is a continuation of the procedure from the lysates prepared in steps 1-4 for both gram positive and gram negative bacterial cultures.

5. **Prepare for binding**
   Add 200 µl of ethanol (95-100%) to each well of HiPurA® 96-well Block (2.2 ml) (LWB-96) 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 HiPurA® 96-well DNA Plate (DBPL-96-01). This precipitate does not interfere with the DNA isolation procedure or with any subsequent applications. Do not use alcohols other than ethanol because this may result in reduced yields.

6. **Load lysate in HiPurA® 96-well DNA Plate**
   Connect the vacuum manifold to the vacuum source. Remove the manifold top and place a HiPurA® 96-well Block (1.6 ml) (LWB-16-96) into the manifold base (to collect the flow-through liquid). Replace the manifold top and place the HiPurA® 96-well DNA Plate (DBPL-96-01) onto the 1.6 ml block. Carefully transfer approximately 700 µl of the lysate from 2.2 ml block and transfer it to the corresponding wells of the HiPurA® 96-well DNA Plate (DBPL-96-01). Place the HiPurA® Silicon Pad to seal (PR11) the plate.

   NOTE: Any Vacuum Manifold, which can accommodate 96-well plates can be used. A negative pressure of -30 inches Hg is required. We recommend the use of a vacuum regulator to adjust the negative pressure. -30 inches Hg is equivalent to approximately 1000 mbar and 15 psi.

7. Turn on the vacuum source and adjust it to -30 inches Hg. Continue to draw vacuum (approximately 10 minutes) through the plates until no liquid remains in any of the wells of the HiPurA® 96-well DNA Plate.

8. Turn off the vacuum source and release the vacuum from inside of the vacuum manifold using the vacuum regulator. Remove the HiPurA® 96-well DNA Plate (DBPL-96-01) from the manifold and temporarily set it aside on a piece of absorbent toweling or plastic wrap.

   NOTE: Discard the flow through liquid from HiPurA® 96-well Block (1.6 ml) (LWB-16-96) and reuse it.

9. **Prewash**
   Add 500 µl of Prewash Solution (PWB) (DS0031) to each well of HiPurA® 96-well DNA Plate (DBPL-96-01) and place the HiPurA® Silicon Pad to seal (PR11) the plate. Turn on the vacuum source and adjust it to -30 inches Hg. Continue to draw vacuum until no Prewash Solution is present in any of the wells. All the flow through will be collected in the bottom collection tube. Discard the flow-through liquid**.**

10. **Wash**
    (Prepare Wash Solution as indicated in General Preparation Instructions)
    Add 500 µl of diluted Wash Solution (WS) (DS0012) to each well of HiPurA® 96-well DNA Plate (DBPL-96-01). Apply vacuum until no Wash Solution is present in any of the wells. Repeat the wash one more time.

11. Discard the flow-through liquid and apply vacuum at a maximum of -30 inches Hg for 10 minutes to remove the traces of ethanol present in the Wash Solution.

12. Turn off the vacuum source. Remove the HiPurA® 96-well DNA Plate (DBPL-96-01) from the vacuum manifold and vigorously tap the plate approximately 6-8 times on several layers of absorbent toweling. Be careful not to damage the drip directors on the underside of the plate.

    NOTE: Lint-free absorbent toweling is recommended to avoid the release of tiny fibres, which could contaminate the DNA and interfere with subsequent downstream applications

13. Incubate HiPurA® 96-well DNA Plate (DBPL-96-01) open without the HiPurA® Silicon Pad for 15 minutes at 70°C in a vacuum oven to evaporate residual ethanol.

    NOTE: If vacuum oven is not available then connect the vacuum manifold to the oven pre-heated at 70°C through the port provided on the oven.

14. **DNA Elution**
    Remove the HiPurA® 96-well Block (1.6 ml) (LWB-16-96), which was used as a collection tray, from the vacuum manifold base and replace it with a new HiPurA® 96-well V-plate (DVB-96) for elution. Reassemble the manifold top and place the HiPurA® 96-well DNA Plate (DBPL-96-01) on the HiPurA® 96-well V-plate. Add 200µl of Elution Buffer (ET) (DS0040) to the corresponding wells of HiPurA® 96-well DNA Plate (DBPL-96-01) and incubate at room temperature (15-25°C) for 5 minutes. Turn on the vacuum source and allow the vacuum (-30 Hg pressure) to continue for 10 minutes.

15. Turn off the vacuum source and use the vacuum regulator to gradually release the vacuum from the manifold. Disassemble the manifold and remove the HiPurA® 96-well V-plate (DVB-96), which contains eluted DNA samples.

Centrifugation based protocol

A. Gram Negative Bacterial Preparation

1. **Harvesting of cells**
   Pellet 1 ml of overnight bacterial broth culture in the HiPurA® 96-well Block (2.2 ml) (LWB-96) by centrifugation at 2250 x g (3,700 rpm) for 10 minutes at room temperature (15-25°C) in a centrifuge with a rotor and carriers/adaptors for 96-well microplates. The block should be covered with HiPurA® Silicon Pad for sealing (PR11) during centrifugation. Remove the medium by gently inverting the block onto a waste container.

NOTE: Tap the inverted block gently onto an absorbent paper towel stack to drain out the remaining droplets of liquid. Ensure that the pellet is firm and does not get disturbed during tapping.

NOTE: If bacteria are grown in rich media such as Terrific broth, it is necessary to reduce the volume of the starting material of the overnight broth culture to 0.5 ml.

2. **Resuspend cells**
   Resuspend each bacterial pellet in 180 µl of Lysis Solution (AL) (DS0015) using multi-channel pipette for easy solution delivery. Mix well by gentle vortexing till no cell clumps are visible.

   NOTE: It is very important that homogenous suspension is obtained as incomplete resuspension will result in poor recovery.

3. **Prepare for cell lysis**
   Add 20 µl of the Proteinase K solution (20 mg/ml) to each well of 96-well plate. Mix and incubate for 30 minutes at 70°C. If residual RNA is not a concern, continue with step 4a.

   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 4a.

4. **Lyse cells**
   Add 200 µl of Lysis Solution (C1) (DS0010), to each well of HiPurA® 96-well Block (2.2 ml) (LWB-96) to lyse the cells. Seal the HiPurA® 96-well Block (2.2 ml) (LWB-96) using the HiPurA® Silicon Pad provided with the kit (PR11). Vortex thoroughly (about 15 seconds) and incubate at 70°C for 10 minutes. Continue with step 5 (Prepare for binding)

   NOTE: A homogeneous mixture is essential for efficient lysis.

B. <u>Gram Positive Bacterial Preparation</u>

Prepare Lysozyme Solution using Lysozyme from chicken egg white (MB098), 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 well. 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.

1. **Harvesting of cells**
   Pellet 1 ml of overnight bacterial broth culture in the HiPurA® 96-well Block (2.2 ml) (LWB-96) by centrifugation at 2250 x g (3,700 rpm) for 10 minutes at room temperature (15-25°C) in a centrifuge with a rotor and carriers/adaptors for 96-well microplates. The block should be covered with breathable tape during centrifugation. Remove the medium by gently inverting the block onto a waste container.

   NOTE: Tap the inverted block gently onto an absorbent paper towel stack to drain out the remaining droplets of liquid. Ensure that the pellet is firm and does not get disturbed during the tapping.

   NOTE: If bacteria are grown in rich media such as Terrific broth, it is necessary to reduce the volume of the starting material of the overnight broth culture to 0.5 ml.

2. **Resuspend cells**
   Resuspend the pellet thoroughly in 200 µl of lysozyme solution (prepared in step 1 of Gram Positive Bacterial Preparation) and incubate for 30 minutes at 37°C.

3. **Prepare for cell lysis**
   Add 20 µl of the Proteinase K solution (20 mg/ml) to each well of 96-well plate. If residual RNA is not a concern, continue with step 4.

   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.

4. **Lyse cells**
   Add 200 µl of Lysis Solution (C1) (DS0010) to each well of HiPurA® 96-well Block (2.2 ml) (LWB-96) to lyse the cells. Seal the HiPurA® 96-well Block (2.2 ml) (LWB-96) using the HiPurA® Silicon Pad provided with the kit (PR11). Vortex thoroughly (about 15 seconds) and incubate at 70°C for 10 minutes. Continue with step 5 (Prepare for binding).

   NOTE: A homogeneous mixture is essential for efficient lysis.

DNA ISOLATION FROM GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

This is a continuation of the procedure from the lysates prepared in steps 1-4 for both gram positive and gram negative bacterial cultures.

5. **Prepare for binding**
   Add 200 µl of ethanol (95-100%) to each well of HiPurA® 96-well Block (2.2 ml) (LWB-96) 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 HiPurA® 96-well DNA Plate (DBPL-96-01). 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.

6. **Load lysate in HiPurA® 96-well DNA Plate**
   Transfer the lysate obtained from step 5 onto HiPurA® 96-well DNA Plate (DBPL-96-01) [placed on HiPurA® 96-well Block (1.6 ml) (LWB-16-96)]. Place the HiPurA® Silicon Pad (PR11) to seal the plate and centrifuge at 3,700 rpm for 5 minutes at room temperature (15-25°C). Discard the flow-through liquid and place the HiPurA® 96-well DNA Plate (DBPL-96-01) in the same 1.6 ml block.

   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 HiPurA® 96-well DNA Plate (DBPL-96-01). 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.

7. **Prewash**
   Add 500 µl of Prewash Solution (PWB) (DS0031) to the HiPurA® 96-well DNA Plate (DBPL-96-01), seal the plate with silicon pad and centrifuge at 3,700 rpm for 5 minutes at room temperature (15-25°C). Discard the flow-through liquid and re-use the same 1.6 ml block.

8. **Wash**
   (Prepare Wash Solution as indicated in General Preparation Instructions)
   Add 500 µl of diluted Wash Solution (WS) (DS0012) to the HiPurA® 96-well DNA Plate (DBPL-96-01), seal the plate with silicon pad and centrifuge for 3 minutes at 3,700 rpm at room temperature (15-25°C). Discard the flow-through and place the HiPurA® 96-well DNA Plate (DBPL-96-01) to the same 1.6 ml block. Centrifuge again at same speed and temperature for the additional 10 minutes to dry the plate.

9. Incubate HiPurA® 96-well DNA Plate (DBPL-96-01) open without the HiPurA® Silicon Pad for 15 minutes at 70°C in a vacuum oven to evaporate any residual ethanol.

   NOTE: If vacuum oven is not available then connect the vacuum manifold to the oven pre-heated at 70°C through the port provided on the oven.

10. **DNA Elution**
    Remove the HiPurA® 96-well Block (1.6 ml) (LWB-16-96) which was used as collection tray and replace it with a new HiPurA® 96-well V-plate (DVB-96) for elution. Place the HiPurA® 96-well DNA Plate (DBPL-96-01) on the HiPurA® 96-well V-plate. Add 200 µl of Elution Buffer (ET) (DS0040) to the corresponding wells of HiPurA® 96-well DNA Plate (DBPL-96-01) and incubate at room temperature (15-25°C) for 5 minutes. Centrifuge at same speed and temperature for 5 minutes to elute the DNA.

    NOTE: 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.

Storage of the eluate with purified DNA: The eluate contains pure bacterial 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® 96 Bacterial DNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Yield	DNA Purity
Bacterial cells	Upto 20 µg/well	1.6-1.9

Trouble shooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Lysozyme is	Solution is	Pipette repeatedly to dissolve the

	difficult to dissolve	inadequately mixed	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.	HiPurA® 96-well DNA Plate 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 plate.
3.	Lysate appears to be gelatinous prior to loading onto the plate	Sample volume is large	Use fewer cells (≤1x 10^{10} cells /ml). The incubation time and or the amount of Proteinase K solution or Lysozyme solution can be increased, depending on which procedure is performed i.e. for Gram positive or Gram negative bacteria. 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 or Lysozyme Solution can be increased, depending on which procedure is performed i.e. for Gram positive or Gram negative bacteria. Eg: The incubation time and the amount of enzyme can be doubled.
		Lysate/Ethanol mixture is not homogenous	Vortex the block or gently mix by pipetting for atleast 5-10 seconds in order to obtain a homogenous solution before applying it to the plate.
		Eluate contains residual ethanol from the wash	Ethanol from the final wash should be eliminated completely before eluting DNA.
		Wash Solution Concentrate was not diluted before use.	Ensure 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 Nuclease and Protease free water should be used for eluting DNA
5.	Purity of the DNA is lower than expected; A_{260}/A_{280} ratio is low	Sample diluted in water	Use either Elution Buffer provided, 10 mM Tris-HCl, 0.5 mM EDTA pH 9.0 or 10 mM Tris-HCl pH 8.0-8.5 as the eluant.
6.	Purity of the DNA is lower than expected; A_{260}/A_{280} ratio is too high.	RNA contamination	RNase A treatment should be included in future isolations or the final product can be treated with RNase A and repurified.
7.	DNA is sheared	Improper handling of genomic DNA	All pipetting steps should be executed as gently as possible. Wide orifice pipette tips are recommended to eliminate shearing of the DNA to a large extent. If the isolated DNA is to be used for PCR, mix with gentle pipetting or invert until homogenous, instead of vortexing, as it reduces shearing of DNA considerably.
		Cells are old	Cells grown for a longer time period may lyse prematurely when subjected to cell wall lysing enzymes, which may result in the release of endogenous nucleases and subsequent DNA degradation.
8.	Downstream applications are inhibited	Traces of ethanol present in the final genomic DNA preparation	Ethanol from the final wash should be eliminated completely before eluting DNA.

Safety Information

HiPurA® 96 Bacterial 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

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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