Wash Solution (HPB)

Product Name	Product Code	Kit Packing
Wash Solution (HPB)	DS0032-250ML	250 ml
	DS0032-500ML	500 ml

Intended Use

Recommended for isolation of DNA from Plasmid.

Introduction: Wash Solution (HPB) is a solution used for the purpose of washing plasmid DNA.

Application: Wash Solution (HPB) is a solution used for the purpose of washing plasmid DNA to be used in molecular biology experiments.

Properties:
Appearance: Colorless solution
Clarity: Clear and free of particles
DNase & RNase: None detected
Suitability Test: This reagent has been tested and is suitable for washing plasmid DNA.

Storage conditions: Wash Solution (HPB) has to be stored at 15 - 25°C. The shelf- life of this solution in 12 months.
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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.

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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

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.

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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

Wash Solution (HPB) 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