MB577 HiPurA® Water DNA Purification Kit (without enrichment)

Kit Contents

Product Code	Reagents provided	MB577		
		20 Preps	50 Preps	250 Preps
DS0202	Water Lysis Solution (WL)	24 ml	60 ml	300 ml
DS0066	Inhibitor Removal Solution (IRSH)	6 ml	15 ml	75 ml
DS0203	Binding Solution (WBS)	16 ml	40 ml	200 ml
DS0012	Wash Solution Concentrate (WS)	4 ml	10 ml	50 ml
DS0204	Wash Solution (WT)	16 ml	40 ml	200 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	2.4 ml	6 ml	30 ml
DBCA020	Hi-Water Bead Tube	20 nos.	50 nos.	250 nos.
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos.	50 nos.	250 nos.
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0 ml)	20 nos.	50 nos.	250 nos.
DBCA017	Collection Tube, Polypropylene (2.0 ml)	60 nos.	150nos.	3 x 250 nos.

Intended Use

Recommended for isolation of DNA from water samples

Introduction

HiPurA® Water DNA Purification Kit (without enrichment) provides a fast and easy method for purification of total DNA from variety of water samples for reliable applications in PCR. 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 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 DNA. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR amplification etc.

HiPurA® Water DNA Purification Kit (without enrichment)

HiPurA® Water DNA Purification Kit (without enrichment) provides a convenient and rapid method for purification of total DNA from variety of water samples. The kit allows rapid isolation and DNA purification procedure using the miniprep spin columns that comprises of three steps viz.

- Adsorption of DNA to the membrane,
- Removal of residual contaminants and
- Elution of pure genomic DNA to yield high quality DNA.

For DNA purification, the water sample is passed through a 0.22 µm filter membrane and the micro-organisms present in the water are trapped. The membrane is then placed in a Hi-Water Bead tube for thorough lysis in presence of a Lysis Buffer. After the inhibitor removal step, the

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DNA is purified using HiElute Miniprep Spin Column (Capped) for further downstream analysis by PCR.

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 present system efficiently couples the speed and reversible nucleic acid-binding properties of the advanced silica gel membrane with versatility of spin column technology to yield larger quantity of DNA with highest purity. 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 such as PCR inhibitors which include divalent cations and proteins are completely removed in two efficient wash steps, leaving pure genomic DNA to be eluted in the buffer provided with the kit.

Elution

The yield of genomic DNA depends on the sample type and the number of cells in the sample. A single elution with 100 µL of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 100 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 genomic DNA respectively. Elution Buffer (ET) is used to dilute samples and to calibrate the spectrophotometer. The absorbance is measured at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should range between 0.1 and 1.0. The 320 nm absorbance is used for correction of 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 $A_{260}–A_{280}$. DNA extracted using HiMedia's HiPurA® Water DNA Purification Kit (without enrichment) 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

- 0.22 µm filter paper
- Filtration system
  HiMedia Product code: SF86 [S.S. Sterility Test Manifold System], SF84 [S.S. Sterility test Filter Holder])
- Vaccum Source (for filtration of water sample)
- Sterile forceps
- Pipettes and sterile tips (barrier / non-barrier) of variable volumes
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Vortex Adapter
- Ethanol (96 – 100%) (For dilution of Wash Solution Concentrate)
- Ice bucket (for 4°C incubation)
- Molecular Biology Grade Water (HiMedia Product code: ML024)

General Preparation Instructions

1. **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.

2. Ensure that clean & dry tubes and tips are used for the procedure.

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

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100%)
20	4 ml	16 ml
50	10 ml	40 ml
250	50 ml	200 ml

Centrifugation

All centrifugation steps are carried out in conventional laboratory centrifuge 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.

Specimen Collection and Handling

Collect water samples in sterile flask. Store the water samples at 15-25°C for short term use or at 2-8°C for long term use.

Types of Specimen: Water samples

Procedure

A. <u>DNA Purification from Water Sample</u>

1. Filter 1000 ml of water sample through a pre-sterilized 0.22 µm filter paper to trap the microorganisms.

2. Aseptically remove the filter paper using sterile forceps, gently roll the paper and place it inside the Hi-Water Bead tube (provided), such that the top side of the filter paper faces inward.

3. **Lysis**
   Add 1 ml of Water Lysis Solution (WL) (DS0202) to the Hi-Water Bead tube. Secure the bead tube horizontally on a vortex adaptor. Vortex at maximum speed for 5 minutes.

4. Centrifuge the Hi-Water Bead Tube (DBCA020) at 5000 rpm for 1 minute (in a 15 ml rotor). Transfer supernatant to a new 2 ml microcentrifuge tube (provided) (approx. 600 – 650 µl of supernatant can be recovered).

   NOTE:
   - This centrifugation step is optional; if not performed, it will result in minor loss of supernatant.
   - The supernatant can be aspirated using a 200µl pipette and placing it at the bottom of the tube to ensure maximum recovery of the lysate.

5. Centrifuge at 13,000 rpm for 1 minute at room temperature (15-25°C). Transfer the supernatant to new 2 ml collection tube (provided) without disturbing pellet.

6. To the above supernatant, add 200 µl of Inhibitor Removal Solution (IRSH) (DS0066), vortex it briefly & incubate at 4°C for 5 minutes.

7. Centrifuge the tube at 13,000 rpm for 1 minute at room temperature (15-25°C). Transfer the supernatant to new 2 ml collection tube (provided) without disturbing pellet.

8. Add 650 µl of Binding Solution (WBS) (DS0203) and mix by vortexing briefly.

9. Load approximately 650 µL of the above solution onto the HiElute Miniprep Spin Column (Capped) and centrifuge for 1 minute at 13,000 rpm at room temperature (15-25°C). Discard the flow-through. Repeat the above step with the remaining sample. Discard the flow-through liquid and reuse the 2.0 ml collection tube (uncapped).

10. Add 650 µl of diluted Wash Solution (WS) (DS0012) to the column and centrifuge at 13,000 rpm for 1 minute. Discard the flow-through, re-use the same 2.0 ml collection tube with column.

11. Add 650 µl of Wash Solution WT (DS0204) to the column and centrifuge at 13,000 rpm for 1 minute. Discard the flow-through and centrifuge again at 13,000 rpm for 2 minutes to dry the column.

12. Place the column in a new 2.0 ml collection tube (uncapped) and add 100 µl of Elution Buffer (ET) (DS0040). Incubate for 5 minutes at room temperature (15-25°C). Centrifuge at 13,000 rpm for 1 minute. Transfer the eluate to a new tube for DNA storage (Store at -20°C).

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® Water DNA Purification Kit (without enrichment) is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Purity
Lake water sample	1.6-1.9

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
2.	HiElute Miniprep Spin Column (Capped) is clogged	Sample volume is large / dense	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.
4.	Poor / Lower yield of genomic DNA	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
		Background reading is high due to silica fines	The DNA sample can be centrifuged at maximum speed for 1 minute, the supernatant can be used to repeat the absorbance readings.
		Sample diluted in water	Use either Elution Buffer provided or 10 mM Tris-HCl, 0.5 mM EDTA pH 9.0 or 10 mM Tris-HCl pH 8.0-8.5 as the eluate.
		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.

		Some water samples have high humic acid content which might inhibit PCR	1. It may be necessary to wash the spin column more than once to reduce humic acid contamination in the final sample. 2. If the DNA recovered at the end of the isolation procedure has a brown color, it may contain humic acid. Dilute DNA 10 fold prior to attempting PCR.
6.	Purity of the DNA is lower than expected; A_{260}/A_{280} ratio is too high.	Traces of ethanol present in the final genomic DNA preparation	After the washing steps, the flow-through 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.
7.	DNA is sheared	Salt is carried over in the final genomic DNA preparation	The column should be transferred to a new capped 2.0 ml collection tube before adding the wash solutions.

Safety Information

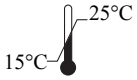
HiPurA® Water DNA Purification Kit (without enrichment) is for laboratory use only, not for drug, household or other uses. The Lysis Solution (C1) contains chaotropic salts, which are irritants. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the 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 at mb@himedialabs.com.



Storage temperature

Do not use if package is damaged

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