MB544 HiPurA® Stool DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB544		
		20 Preps	50 Preps	250 Preps
DS0086	TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0)	30 ml	75 ml	375 ml
DS0015	Lysis Solution (AL)	12 ml	30 ml	150 ml
DS0085	Stool Lysis Buffer (SL1)	6 ml	15ml	75 ml
DS0066	Inhibitor Removal Solution (IRSH)	8 ml	20 ml	100 ml
DS0019	Wash Solution Concentrate (WSP)	12 ml	30 ml	150 ml
DS0067	Binding Solution (SB)	6 ml	15 ml	75 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	6 ml	15 ml	75 ml
MB086	Proteinase K	10 mg	25mg	125 mg
DS0003	RNase A Solution (20 mg/ml)	0.5 ml	1.25 ml	6.25 ml
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
PW1139	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

Intended Use

Recommended for isolation of DNA from human and animal stool samples.

Introduction

HiPurA® Stool DNA Purification Kit provides a fast and easy method for purification of stool DNA for reliable applications in PCR and Southern blotting technique, 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 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 is obtained from various species. The purified DNA obtained is compatible with downstream applications such as restriction enzyme digestion, hybridization techniques, PCR and Southern blotting.

HiPurA® Stool DNA Purification Kit

This HiPurA® Stool DNA Purification Kit simplifies isolation of DNA from stool using spin column technique. The procedure is optimized for rapid and reliable isolation of high-quality total DNA from upto 250 mg of fresh or frozen stool samples. Stool samples possess typically many compounds that can degrade DNA and inhibit downstream enzymatic procedures. The HiPurA® Stool DNA Purification Kit contains a unique solution, Inhibitor Removal Solution (IRSH) (DS0066) to remove these inhibitory compounds at an early stage during the extraction process.

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The total genomic DNA obtained is bound to silica membrane in a spin column format, washed and eluted from the membrane. The purified DNA can directly be used for PCR analysis and other downstream applications.

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.

Elution

The yield of genomic DNA depends on the sample type and the amount of flora present in the sample. A single elution with 200 µl of Elution Buffer (ET) (DS0040) or Molecular Biology Grade Water (ML024) will provide sufficient DNA to carry out multiple amplification reaction. The eluted DNA is suitable for direct use in PCR, restriction digestion, and hybridization techniques.

Concentration, yield and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer (ET) or Molecular Biology Grade Water (ML024) 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® Stool 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

- Ethanol (96-100 %)
- Tabletop Microcentrifuge (capable of atleast 13,000 x g {≈14,000 rpm})
- Molecular Biology Grade Water (ML024)
- Water bath or heating block preheated to 55°C, 70°C

Storage

Store the HiPurA® Stool DNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 1 year.

General Preparation Instructions

1. Preheat a water bath or heating block to 55°C, 70°C.

2. **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.

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

4. **Dilute Wash Solution Concentrate (WSP) (DS0019) as follows:**

Number of Preps	Wash Solution Concentrate (WSP)	Ethanol (96-100 %)
20	12 ml	28 ml
50	30 ml	70 ml
250	150 ml	350 ml

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

   The HiPurA® Stool 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
20	10 mg	0.5 ml
50	25 mg	1.25ml
250	125 mg	6.25 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.

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 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 Handling and Collection

Collect stool sample in a sterile container (if to be used for future) and store the samples at 2-8°C for short term storage or -20°C for long term storage. Ensure that the stool sample is at room temperature (15-25°C) before beginning the protocol. After use, contaminated material must be sterilized by autoclaving before discarding.

Types of Specimen

Clinical samples: Stool sample

Procedure

1. **Resuspension:**
   Take 250 mg of stool sample, add 1ml of TE buffer (DS0086). Vortex vigorously and centrifuge at ≥8,000 x g (≥10,000 rpm) for 3 minutes. Discard the supernatant.

2. Resuspend the pellet thoroughly in 500 µl of Lysis Solution (AL) (DS0015). Pipet out 200 µl of the resuspended solution in a new 2ml capped collection tube and prepare for lysis.

3. **Lysis**
   To 200 µl of resuspended solution, add 20 µl of the Proteinase K Solution (20 mg/ml) **(Refer General Preparation Instructions)**. Mix by vortexing and incubate for 30 minutes at 55°C. If residual RNA is not a concern, continue with step 4.

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

5. **Lysis**
   Add 200 µl of Stool Lysis Buffer (SL1) (DS0085), vortex thoroughly (about 15 seconds), and incubate at 70°C for 10 minutes.
   NOTE: A homogeneous mixture is essential for efficient lysis.

6. **Inhibitor removal**
   Add 250 µl of Inhibitor Removal Solution (IRSH) (DS0066), vortex for few seconds and incubate at 4°C for 5 minutes.

7. Centrifuge the tube for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature.

8. **Binding**
   Transfer the supernatant to a clean collection tube (2.0 ml) (not provided), add 200 µl of Binding Solution (SB) (DS0067) and vortex for few seconds.

9. **Load onto HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Load the lysate on the HiElute Miniprep Spin Column (capped) and centrifuge for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature. Discard the flow-through.

10. **Wash**
    (Prepare Wash Solution as indicated in General Preparation Instructions)
    Add 500 µl of diluted Wash Solution (WSP) (DS0019) and centrifuge at 10,000 x g (≈ 12,000 rpm) at room temperature for 1 minute. Discard the flow-through. Repeat the wash step one more time.

11. Discard the flow-through and centrifuge the HiElute Miniprep Spin column (Capped) at 10,000 x g (≈12,000 rpm) at room temperature for an additional 1 minute to remove any residual ethanol.

12. **DNA Elution**
    Transfer the column to a fresh uncapped collection tube 2.0 ml and add 200 µL of Elution Buffer (ET) (DS0040) or Molecular Biology Grade Water (ML024) directly onto the center of the column membrane. Centrifuge the tube for 1 minute at 10,000 x g (≈ 12,000 rpm) at room temperature.

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

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 denaturation of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

Warning and Precautions

For Laboratory and Research Use only. Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical 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

Performance of the kit is expected when the kit is used as per the protocol mentioned in the product insert within the expiry period when stored at recommended temperature.

Quality Control

Type of Sample	DNA Yield	DNA Purity
Stool sample	Upto 10 µg	1.6-1.9

Troubleshooting guide

Sr. No.	Problem	Possible Cause	Solution
1	Purity of the DNA is lower than expected (A_{260}/A_{280} ratio is less)	Insufficient elimination of inhibitory compounds	Repeat the DNA isolation with a new sample. Be sure to mix the sample with Inhibitor Removal Solution (IRSH) (DS0066) thoroughly.
		No ethanol added to the Wash Solution Concentrate (WSP).	Dilute the Wash Solution Concentrate (WSP) (DS0019) with ethanol (96-100%) as mentioned in General Preparation Instructions.
2	A_{260}/A_{280} ratio is too high	RNA contamination.	Ensure that the sample is treated with RNase A Solution as mentioned in the protocol.

3	Low genomic DNA yield or no DNA eluted	Poor homogenization of sample	Repeat the DNA isolation with a new sample. Be sure to mix the sample with TE buffer.
		DNA got washed off	Dilute Wash Solution Concentrate (WSP) (DS0019) by adding appropriate volume of ethanol (96-100%) as mentioned in General Preparation Instructions.
4	Poor performance of DNA in downstream experiments	Ethanol carryover	Ensure that the column is centrifuged for an additional minute at maximum speed as mentioned in the protocol. Following the spin, remove the column carefully from the collection tube so that it does not come in contact with the flow-through as this will result in carryover of ethanol.
		High concentration of DNA inhibits PCR reaction	Dilute the DNA eluate used in downstream applications.
		No specific bands observed in downstream PCR	Use hot-start Taq Polymerase mixture
		Inhibitory substances present in eluted DNA	Check the A_{260}/A_{280} ratio and dilute the eluate to 1:50 if necessary
5	Clogged HiElute Miniprep Spin column (Capped)	Insufficient centrifugation	The g-force and the centrifugation time can be increased.
6	Little or no supernatant observed after initial centrifugation	Insufficient centrifugal force	The g-force and centrifugation time can be increased

Safety Information

The HiPurA® Stool 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. 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.

Please refer disclaimer Overleaf.

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.

IVD	In vitro diagnostic medical device
CE	CE Marking
25°C 15°C	Storage temperature
	Do not use if package is damaged
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