

MB543

HiPurA® Fungal DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB543		
		20 Preps	50 Preps	250 Preps
DS0016	Lysis Buffer (PL)	10 ml	25 ml	125 ml
DS0017	Precipitation Buffer (PS)	4 ml	10 ml	50 ml
DS0018	Binding Buffer Concentrate (BB)	16ml	40 ml	200 ml
DS0019	Wash Solution Concentrate (WSP)	12 ml	30 ml	150 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	6 ml	15 ml	75 ml
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]	20nos	50 nos	250 nos
DSCA02	HiShredder (in DBCA016 Collection Tube)	20 nos	50 nos	250 nos
DBCA016	Collection Tubes (Uncapped) , Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
DBCA017	Collection Tubes, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

Intended Use

Recommended for isolation of DNA from Fungal samples.

Introduction

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

HiPurA® Fungal DNA Purification Kit

This kit simplifies isolation of DNA from fresh fungal material with spin column procedure. The procedure is optimized for a maximum of 100-150 mg of wet-weight starting material. The sample is ground in liquid nitrogen along with Lysis Buffer (PL). Protein precipitation is followed by removal of other contaminants using HiShredder. The flow-through fraction is then mixed with a solution that enhances the binding of DNA to the column. The solution is then passed through HiElute Miniprep Spin Column (Capped) that is followed by washing steps to remove trace contaminants. High quality DNA is eluted in the Elution Buffer (ET) provided in the kit. Typical yield from 100-150 mg of wet weight sample is 2-30 µg (depending on the species).

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 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. The purified DNA is upto 20-30 kb in length and can be used for further downstream applications.

HiShredder (DSCA02)

For the HiMedia's HiPurA® Fungal DNA Purification procedure, contaminants such as cell debris, salt precipitates are removed by centrifugation through a HiShredder. The HiShredder removes all cell debris and precipitates making the preparation of a clear lysate rapid and efficient. Preparation of the clear lysate is essential to prevent clogging of the HiElute Miniprep Spin Column (Capped) used in the subsequent steps.

Elution

The yield of fungal DNA depends on the sample type and the number of cells in the sample. 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 upto 20-30 kb and is suitable for direct use in PCR, restriction digestion and Southern blotting applications etc.

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) 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® Fungal 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

- Small mortar and pestle
- Liquid nitrogen
- Tabletop Microcentrifuge (with rotor for 2.0 mL tubes)
- 65°C water bath or heating block

Storage

Store the HiPurA® Fungal DNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 18 months.

General Preparation Instructions

1. Preheat a water bath or heating block to 65°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 Binding Buffer Concentrate (BB) (DS0018) as follows:**

Number of Preps	Binding Buffer Concentrate (BB)	Ethanol (96-100%)
20	16 ml	8 ml
50	40 ml	20 ml
250	200 ml	100 ml

5. **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

6. Prechill the mortar and pestle 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 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 (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.

Specimen Collection and Handling

Collect fungal tissue in a sterile container and store the sample at 2-8°C for short term storage or -20°C for long term storage.

Types of Specimen

Samples: Fungus

Sample preparation

A. Growing and Harvesting Cells from Liquid Cultures

Medium: Potato Dextrose Broth (M403)

Sabouraud Dextrose Broth (Sabouraud Liquid Medium-M033)

To 100 mL of the medium (Potato Dextrose broth or Sabouraud Dextrose Broth) add 10 µl of Tween 80 (RM159) to the medium before autoclaving (at 115°C for 15 minutes). Tween 80 keeps the fungal cells dispersed and helps in weighing the culture. After inoculation of fungal culture, incubate at 30°C, preferably on a shaker. It is preferable to use wet fresh fungal tissue, since they contain more cells per weight and therefore result in higher yields.

Fungal walls consist of complex of fibrillar material embedded in polysaccharide that enables the proteins present to make the wall a functional unit. The wall may be highly protected or relatively susceptible to the environment because of various constituents in the matrix. Therefore, extraction of DNA from fungal cultures is difficult and the yield varies with different species.

B. Manual Disruption with Mortar and Pestle

It is advisable to use rough mortar and pestle with rough grinding surfaces (rather than smooth agate mortar and pestle) as fungal tissue is a hard and sticky mass, difficult to grind. Grind the weighed fungal tissue using a mortar and pestle in liquid nitrogen to a fine powder. Allow the liquid nitrogen to evaporate. **DO NOT ALLOW THE SAMPLE TO THAW** (Keep samples on ice if needed). Proceed immediately to the DNA isolation protocol.

NOTE: Delay in continuing to DNA isolation after sample preparation will result in DNA degradation and low yield.

DNA isolation protocol

1. To a maximum of 100-150 mg of fungal tissue that has been already disrupted by grinding, add 400 µl of Lysis Buffer (PL) (DS0016). Transfer the mixture to a 2 ml capped centrifuge tube provided and add 20 µl of RNase A Solution (20 mg/mL) (DS0003). Vortex vigorously.

NOTE: Do not mix Lysis Buffer (PL) and RNase A before use.

2. Incubate the mixture for 10 minutes at 65°C, mix the contents 2-3 times by inverting the tube.
3. Add 130 µl of Precipitation Buffer (PS) (DS0017) to the lysate, mix and incubate for 5 minutes on ice.

Optional: Centrifuge the lysate for 5 minutes at ≈13,000 rpm.

NOTE: Certain fungal species can generate viscous lysates with large amounts of precipitates due to variation in water and polysaccharide content, resulting in shearing of DNA in the next step. For optimal results, the precipitate can be removed by centrifugation at ≈13,000 rpm for 5 minutes. After centrifugation, add the supernatant to the HiShredder.

4. **Load lysate in HiShredder (DSCA02)**

Add the lysate to the HiShredder placed in a 2.0 mL uncapped collection tube and centrifuge for 2 minutes at ≈ 13,000 rpm.

5. Transfer the flow-through fraction from step 4 to a 2.0 mL collection tube (not provided) without disturbing the cell debris pellet.

6. **Binding**

(Prepare the Binding Buffer as indicated in General Preparation Instructions)

Add 1.5 volumes of diluted Binding Buffer (BB) (DS0018) to the cleared lysate and mix by pipetting.

NOTE: E.g.: To 450 µl of lysate add 675 µl of diluted Binding Buffer (BB). The volume of buffer can be reduced accordingly if less lysate is obtained. A precipitate may form after the addition of ethanol but this will not affect the DNA isolation procedure.

7. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
Add 650 µl of the mixture from step 6, including any precipitate which may have formed to the HiElute Miniprep Spin Column (Capped) placed in a 2.0 mL collection tube. Centrifuge for 1 minute at 6000 x g (≈8000 rpm). Discard the flow-through.
8. Repeat step 7 with the remaining sample (if any). Discard the flow-through liquid and the 2.0 mL collection tube.
9. **Wash**
(Prepare the Wash Buffer as indicated in General Preparation Instructions)
Place the column in a same collection tube add 500 µl of diluted Wash Buffer and centrifuge for 1 minute at 6000 x g (≈8000 rpm).
NOTE: Discard the flow-through and reuse the 2.0 mL uncapped collection tube in step 10.
10. Add another 500 µl of the diluted Wash Buffer (WSP) to the column and centrifuge for 1 minute at ≈13,000 rpm. Discard the flow-through and reuse the same collection tube.
11. Centrifuge the tube with column for an additional 2 minutes at a maximum speed ≈13,000 rpm) to dry the membrane.
12. **DNA Elution**
Place the column in a new 2.0 mL uncapped collection tube and pipette 100 µl of the Elution Buffer (ET) (DS0040) directly into the column without spilling to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute to elute the DNA. Repeat the elution step again with another 100 µl of Elution Buffer (ET) for high yield of DNA.

NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer (ET), then centrifuge. DNA elution can also be performed in single step by the addition of 200 µl of Elution Buffer (ET) at a time (However, DNA yield would be low). Storing DNA in water can cause acid hydrolysis.
13. Transfer the eluate to a fresh capped 2mL collection tube for longer storage.

Storage of the eluate with purified DNA: The eluate contains pure genomic DNA. For short-term storage (24-48 hours) 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 (ET) 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® Fungal DNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Yield	DNA Purity
Fungal sample (100 mg)	2-30 µg of DNA	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. Birren, B. and Lai, E. Pulsed Field Gel Electrophoresis: A practical guide (Academic Press, San Diego, CA, 1993).

Troubleshooting guide

Sr. No.	Problem	Possible Cause	Solution
1.	Clogged HiShredder	High viscosity of the lysate	Perform the optional centrifugation step as mentioned in step 3 of the protocol before loading a large amount of the lysate onto the HiShredder.
		Insufficient centrifugation	The g-force and the centrifugation time can be increased.
2.	Clogged HiElute Miniprep Spin column	Carryover of the particulate material	Ensure that no particulate material is transferred following centrifugation through the HiShredder.
		Lysate is too viscous	The amount of starting material can be reduced or the amount of buffers PL and PS can be increased.
		Insufficient centrifugation	The g-force and the centrifugation time can be increased.
3.	Lower yields of DNA	Insufficient disruption of the fungal tissue	Ensure that the fungal tissue is disrupted in sufficient amounts of liquid nitrogen. It is very important that the disrupted tissue sample should not thaw before addition of Lysis Buffer.
		Insufficient lysis	The amount of the starting material can be reduced or the amount of buffers PL and PS can be increased.
		Incorrect binding	Ensure that the binding conditions are adjusted correctly by accurately determining the amount of lysate recovered (as per step 6).
		DNA still bound to the membrane	The volume of Elution Buffer (ET) or water can be increased upto 200 µl and tubes can be incubated at room temperature (15-25°C) for 5 minutes before centrifugation.

4.	Shearing of DNA	Precipitation of Lysis Buffer (PL)	Examine the solution for any kind of precipitation. If the 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.
		Presence of debris and precipitation of lysate	The optional centrifugation step can be performed before loading a large amount of lysate onto HiShredder.
5.	Darkly coloured membrane or green/yellow eluate after washing with wash solution	Insufficient washing of the membrane	After washing with the Wash Buffer (WSP), an additional wash with 500 µl ethanol (96-100%) should be performed. Centrifuge for 2 minutes at 13,000 x g (≈14,000 rpm) to dry the membrane.
		Amount of starting material is more than recommended	Reduce the amount of starting material for future preps.
6.	Poor performance of DNA in downstream experiments	Ethanol carryover	Ensure that after the second wash with Wash Buffer (WSP), the column is centrifuged for 2 minutes at 13,000 x g (≈14,000 rpm). 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.
		Salt carryover	Ensure that the Wash Buffer is at room temperature (15-25°C) before use.

Safety Information

HiPurA® Fungal 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.

Symbols

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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Disclaimer :

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