MB552 HiPurA® Yeast Genomic DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB552		
		20 Preps	50 Preps	250 Preps
DS0015	Lysis Solution (AL)	6 ml	15 ml	75 ml
DS0010	Lysis Solution (C1)	6 ml	15 ml	75 ml
DS0031	Prewash Solution (PWB)	12 ml	30 ml	150 ml
DS0012	Wash Solution Concentrate (WS)	4 ml	10 ml	50 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	4 ml	10 ml	50 ml
MB086	Proteinase K	12 mg	30 mg	150 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 yeast cultures

Introduction

HiPurA® Yeast Genomic DNA Purification Kit provide a fast and easy method for purification of total DNA for reliable applications in PCR, 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 DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR amplification and Southern blotting.

HiPurA® Yeast Genomic DNA Purification Kit

This kit simplifies isolation of DNA from yeast by the spin-column procedure. Yeast cells (*Saccharomyces cerevisiae*, *Candida albicans*), are grown in log phase and spheroplasts are subsequently prepared. Following lysis, the DNA is bound to the silica-gel membrane of the HiElute Miniprep Spin Column. 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.

HiElute Miniprep Spin Column (Capped) [DBCA02]

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.

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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.

Elution

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

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® Yeast 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

- 30°C water bath or heating block
- 56°C water bath or heating block
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96-100%)
- 2- mercaptoethanol (ß -ME) (Product Code: MB041)
- Zymolyase or Lyticase (Product Code: MB099)
- YPD broth for *Saccharomyces cerevisiae* (Product code: M1363) or Candida Medium for *Candida* species (Product Code: M104) can be used
- Sorbitol (Product Code: MB066) Buffer (Refer 'General Preparation Instructions' below)
- Molecular Biology Grade Water (Product code: ML024)

Storage

Store the HiPurA® Yeast Genomic 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 30°C.
2. Preheat a water bath or heating block to 56°C.
3. Preset the centrifuge at 4°C (for initial steps of the protocol).

4. **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.

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

6. **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

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

   The HiPurA® Yeast 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
20	12 mg	0.6 ml
50	30 mg	1.5 ml
250	150 mg	7.5 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.

8. **Prepare Sorbitol buffer as follows:**
   1M sorbitol
   100 mM EDTA

   Just before use, add:
   10 µl of ß-mercaptoethanol per 1 ml of Sorbitol Buffer.

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.

NOTE: If the isolated DNA is to be used for PCR, mix with gentle pipetting or inversion until homogenous, instead of vortexing in the following procedure as it reduces shearing of DNA considerably.

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: Yeast culture

Procedure

1. Grow yeast culture *Saccharomyces cerevisiae* or *Candida spp.* in YPD medium (Product Code: M1363). Harvest cells, maximum up to 1 X 10^8 or up to 1.5ml of overnight grown yeast culture in capped 2ml centrifuge tube by centrifuging at 1500 rpm for 5 minutes at 4°C. Remove the culture medium completely and discard.

2. **Resuspend cells**
   Resuspend the pellet in 600 µl of Sorbitol Buffer **(Refer General Preparation Instructions)**. Add 50U of zymolyase or lyticase and incubate at 30°C for 30 minutes.

3. Pellet the spheroplasts by centrifuging for 10 minutes at 6500 x g (10,000 rpm) at 4°C. Discard the supernatant without disturbing the pellet.

4. **Lyse cells**
   Resuspend the spheroplasts in 180 µl of Buffer AL (DS0015).

5. Add 25 µl of the Proteinase K solution (20 mg/ml) **(Refer General Preparation Instructions)** to the sample. Mix and incubate for 30 minutes at 56°C. If residual RNA is not a concern, continue with step 7.

6. **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 7.

7. **Lyse cells**
   Add 200 µl of Lysis Solution (C1) (DS0010), vortex thoroughly (about 15 seconds) and incubate at 56°C for 10 minutes.

   NOTE: A homogeneous mixture is essential for efficient lysis.

8. **Prepare for binding**
   Add 200 µl of ethanol (95-100%) to the lysate 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 HiElute Miniprep Spin Column. 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.

9. **Load lysate onto HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Transfer the lysate obtained from step 8 onto HiElute Miniprep Spin Column (Capped) provided. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and place the spin column in same 2.0 ml collection tube.

   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 column. 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.

10. **Prewash**
    Add 500 µl of Prewash Solution (PWB) (DS0031) to the column and centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

11. **Wash**
    (Prepare Wash Solution Concentrate (WS) as indicated in General Preparation Instructions)
    Add 500 µl of diluted Wash Solution (WS) (DS0012) to the column and centrifuge for 3 minutes at maximum speed 12,000-16,000 x g (≈13,000-16,000 rpm). Give an additional spin at same speed for the 1 minute to dry the column. The column must be free of ethanol before eluting the DNA.

12. **DNA Elution**
    Transfer the column to new uncapped collection tube. Pipette 50 µl of the Elution Buffer (ET) (DS0040) directly into the column without spilling to the sides. Incubate for 1 minute at room temperature. Centrifuge at ≥6,500 x g (≈ 10,000 rpm) for 1 minute to elute the DNA. Repeat the elution step one more time in the same collection tube.

    NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Buffer (ET), then centrifuge. Elution with volumes less than 100 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.

13. Transfer the eluate to a fresh capped 2ml collection tube for long term 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 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® Yeast Genomic DNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Purity
S. cerevisae	1.6-1.9

Troubleshooting Guide:

Sr.No.	Problem	Possible Cause	Solution
1.	HiElute Miniprep Spin Column (Capped) 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 spin column.
2.	Lysate appears to be gelatinous prior to loading onto the column	Sample volume is large	Use fewer cells (≤1 x 10^{10} cells /ml). The incubation time and/or the amount of Proteinase K solution can be increased. Eg: The incubation time and the amount of enzyme can be doubled.
3.	Poor / Lower yield of genomic DNA	Sample is old	It is necessary to use cells before they reach their maximum density.
		Incomplete lysis of cells	The incubation time and/or the amount of Proteinase K solution can be increased. Eg: The incubation time and the amount of enzyme can be doubled.
		Lysate/Ethanol mixture is not homogenous	Vortex the tubes for atleast 5-10 seconds in order to obtain a homogenous solution before applying it to the column.
		DNA elution is incomplete. Eluate contains residual ethanol from the wash	1. 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. 2. 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)
4.	Purity of the DNA is lower than expected; A_{260}/A_{280} ratio is low	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 eluant.
5.	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.
6.	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 inverting 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.
7.	Downstream applications are inhibited	Traces of ethanol present in the final genomic DNA preparation	After the washing steps, the eluate 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.

Please refer disclaimer Overleaf.

Safety Information

The HiPurA® Yeast Genomic DNA Purification Kit 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 to mb@himedialabs.com.

Storage temperature

Do not use if package is damaged

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