MB552MPF16 HiPurA® Pre- filled Plates for Yeast DNA Purification

Kit Contents

Product Code	Reagents provided	MB552MPF16
		96 PR
PF16O1	Pre-filled Plates for Yeast DNA Purification	6 no
LA1118B	Magnetic Rod Tip	12 no
DS1005A	Magnetic Beads	2 ml
DS0003	RNase A (20mg/ml)	2 ml
DS0013	Proteinase K	2.5 ml
DS0015	Lysis Solution (AL)	18 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH8.5]	7 ml

Intended Use

Recommended for isolation of DNA from yeast cultures.

Introduction

HiPurA® Pre- filled Plates for Yeast DNA Purification for Insta NX® Mag16 provides the fastest and easiest way to purify DNA for reliable use in amplification technologies. HiPurA® Pre- filled Plates for Yeast DNA Purification can be used for isolation of DNA from yeast samples, but the performance may vary depending on the sample type.

HiPurA® Pre- filled Plates for Yeast DNA Purification

This kit carries out efficient extraction of genomic DNA from different samples. Yeast cells (*Saccharomyces cerevisiae*, *Candida albicans*), are grown in log phase and spheroplasts are subsequently prepared. The DNA purification procedure comprises of three steps viz. adsorption of DNA to the magnetic beads, removal of residual contaminants and elution of pure genomic DNA. The magnetic beads have a high binding capacity and high-quality genomic DNA is obtained from various species. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

Elution

The yield of DNA depends on the sample type and the number of cells in the sample. A single elution with Elution Solution will provide sufficient DNA to carry out multiple amplification reactions.

Storage

HiPurA® Pre- filled Plates for Yeast DNA Purification can be stored at room temperature (15-25°C) for up to 2 years without showing any reduction in performance. We advise a certain storage temperature for the reagents listed below:

- **On receipt store Proteinase K (DS0013): at -20°C.**
- **On receipt store RNase A (DS0003): at 2-8°C.**
- **On receipt store Magnetic Beads (DS1005A): at 2-8°C.**

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Specimen Handling and Collection
Collect overnight culture from sterile flask with the help of micropipette. Store the remaining culture at 2-8°C for short term use.

Type of Specimens
Yeast culture

Materials needed but not provided
1. 30°C water bath or heating block
2. 56°C water bath or heating block
3. Centrifuge at 4°C (with rotor for 2.0 ml tubes)
4. Ethanol (96-100%)
5. 2- Mercaptoethanol (ß -ME) (Product Code: MB041)
6. Zymolyase or Lyticase (Product Code: MB099)
7. YPD broth for *Saccharomyces cerevisiae* (Product code: M1363) or Candida Medium for *Candida* species (Product Code: M104) can be used
8. Sorbitol (Product Code: MB066) Buffer (Refer 'General Preparation Instructions' below)
9. 100mM EDTA
10. Molecular Biology Grade Water (Product code: ML024)
11. HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)

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
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. **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.
7. Vortex magnetic beads before use.

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).

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 10 µl of zymolyase or Lyticase (not provided) 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 (DS0013) to the sample. Mix and incubate for 30 minutes at 56°C. If residual RNA is not a concern, continue with the addition of sample to the plate.

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 with the addition of sample to the plate. **This will be your pre- processed sample.**

Set up processing Plates as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Open the door of Insta NX® Mag16 machine.
3. Select **"MB55216"** program.
4. Remove the seal from the Pre-filled Plate for Yeast DNA Purification (PF16O1).

   NOTE: Take care while peeling off the seal. Hold the plate firmly by your one hand and then peel off the seal by holding it in your other hand without shaking.

5. Add **50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th and 12th column of the Pre-filled Plates for Yeast DNA purification (PF16O1).**
6. **Add 200µl of pre- processed sample and 20µl of Magnetic Beads (DS1005A) in the 1st and 7th column** of the Pre-filled Plate for Yeast DNA purification (PF16O1).
7. After adding the above solutions place the Plates on the platform.
8. Place the Magnetic Rod Tip (LA1118B) by sliding onto the machine.

   NOTE: After placing the rods ensure that the rods are properly fixed on their place.

9. Close the door of Insta NX® Mag16 machine.
10. Click on the RUN option on the home screen.
11. After the run is complete, remove the Pre-filled Plate for Yeast DNA Purification from the position. Discard the Magnetic Rod Tip (LA1118B). Dispense the eluted DNA from column 6 and 12 to a new HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017) (not provided). The eluate contains pure DNA.

NOTE: If small amount of magnetic beads are observed in the final eluate then keep the cartridges along with cartridge holder on Magnetic pad (not provided) for 4-5 minute and collect supernatant carefully without disturbing beads pellet in new collection tube.

Storage of the eluate with purified DNA: The recommended storage temperature for the eluted DNA is -80°C. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA.

Warning

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

The yield of DNA depends upon the type and the volume of starting material used.

Performance and Evaluation

The yield and efficiency of purification is determined by performing Polymerase Chain Reaction.

Quality Control

Each lot of HiMedia's HiPurA® Pre- filled Plates for Yeast DNA Purification is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre- filled Plates for Yeast DNA Purification is for laboratory use only, not for drug, household or other uses. 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.

Please refer disclaimer Overleaf.

Symbols

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

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Disclaimer :

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