

MB571MPF16

HiPurA® SuperPlant Pre- filled Plates for Plant DNA Extraction

Kit Contents

Product Code	Reagents provided	MB571MPF16
		96 PR
PF16Y	SuperPlant Pre-filled Plates for Plant DNA Extraction	6 no
DS0200A	SuperPlant Extraction Buffer A	87 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	12 ml
DS0003	RNase A (20mg/ml)	2 ml
DS1005A	Magnetic Beads	2 ml
DS0070	Additive-II	9 ml
DS0071	Additive-III	1.8 g
LA1118B	Magnetic Rod Tip	12 no

Intended Use

Recommended for isolation of DNA from plant samples.

Introduction

HiPurA® SuperPlant Pre- filled Plates for Plant DNA Extraction for Insta NX® Mag16 provides the fastest and easiest way to purify DNA for reliable use in amplification technologies. HiPurA® SuperPlant Pre- filled Plates for Plant DNA Extraction can be used for isolation of DNA from various samples, but the performance may vary depending on the sample type. The DNA purification procedure comprises of three steps viz. adsorption of DNA to the magnetic beads, removal of residual contaminants and elution of pure DNA. The magnetic beads have a high binding capacity and high-quality DNA is obtained from sample. The purified DNA can directly be used for PCR analysis and other downstream applications.

HiPurA® SuperPlant Pre- filled Plates for Plant DNA Extraction

This kit carries out efficient extraction of DNA from plant samples. The procedure is optimized for a maximum of 200 mg of wet-weight of the starting material. The sample is cut and ground in liquid nitrogen or without liquid nitrogen along with SuperPlant Extraction Buffer A. The flow-through fraction is then mixed with a solution that enhances the binding of DNA to the magnetic particles. The magnetic particles are then carried forward to the washing step to remove trace contaminants. High quality DNA is eluted in the Elution Buffer (ET) provided in the pre-filled Cartridges.

Elution

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

Storage

HiPurA® SuperPlant Pre- filled Plates for Plant DNA Extraction 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 RNase A (DS0003): at 2-8°C.**
- **On receipt store Magnetic Beads (DS1005A): at 2-8°C.**

Materials needed but not provided

- Small mortar and pestle
- Liquid nitrogen (Optional)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes) capable of upto $\approx 13,000$ rpm [TabSpin® 012 (LA1090)]
- 65°C water bath or heating block
- Insta NX® Mag16 (Product Code: LA1096)
- Vortex
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)
- Chloroform: Isoamylalcohol (24:1) (Product Code: MB115)

General Preparation Instructions

1. Grinding of the plant material can be done using mortar and pestle. Midrib should be removed from the material before grinding, as midrib is a major source of carbohydrate contamination.
2. **SuperPlant Extraction Buffer:** Immediately prior to use, add 90 μ l of Additive-II (DS0070) and 18 mg of Additive-III (DS0071) in 900 μ l of SuperPlant Extraction Buffer (DS0200A). Preheat the solution to 65°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 260nm 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).

Specimen Handling and Collection

Sample Preparation with liquid Nitrogen

Finely cut the leaf material before grinding. Weigh 200 mg of the finely cut plant material and grind properly 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.

OR

Sample Preparation without liquid Nitrogen

Finely cut the leaf material before grinding. Weigh 200 mg of the finely cut plant material and grind properly using a mortar and pestle 900 µl of SuperPlant Extraction Buffer A (DS0200A) (preheated to 65°C) (Refer General Preparation Instructions). **Proceed immediately to the DNA isolation protocol with step 2.**

Pre- processing of samples

NOTE: Ensure that Additive-II (DS0070) and Additive-III (DS0071) are added to SuperPlant Extraction Buffer A (DS0200A) as mentioned in General Preparation Instructions.

1. To 200 mg of the ground material (with liquid nitrogen) add 900 µl of SuperPlant Extraction Buffer A (DS0200A) (preheated to 65°C) (**Refer General Preparation Instructions**) and transfer the sample to a capped 2.0 ml collection tube (not provided). Mix by vortexing.
2. Incubate the samples for 60-90 minutes with occasional inversion at 65°C.
3. Add 1 ml of Chloroform: Isoamylalcohol (24:1) (not provided) and mix gently by inversion for 5 minutes.
4. Centrifuge the samples at 13,000 x g [≈14,000 rpm] for 10 minutes at room temperature (15-25°C).
5. Transfer the top aqueous layer (containing DNA) into a new 2.0ml collection tube (not provided) and add 20 µl of RNase A Solution (20 mg/ml) (DS0003). Incubate for 5 minutes at room temperature (15-25°C). **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. Select “**MB57116**” program. Open the door of the Insta NX® Mag16 machine.
3. Remove the seal from the **SuperPlant Pre-filled Plate for Plant DNA Extraction (PF16Y)**.

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

4. Add **100µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th and 12th column of the SuperPlant Pre-filled Plate for Plant DNA Extraction (PF16Y)**.
5. Add approximately **450µl pre- processed sample** in the **1st and 2nd column** and for another sample add approximately **450µl pre- processed sample** in **7th and 8th column of the SuperPlant Pre-filled Plate for Plant DNA Extraction (PF16Y)**.

NOTE: Column 1st, 2nd of Pre-filled Plates for SuperPlant Pre-filled Plate for Plant DNA Extraction (PF16Y) should contain same sample material. Similarly, Column 7th, 8th of Pre-filled Plates for SuperPlant Pre-filled Plate for Plant DNA Extraction (PF16Y) should contain same sample material. Final volume of a single sample should be 450µl.

6. Add **10 µl Magnetic Beads (DS1005A)** in the **1st & 2nd and the 7th & 8th column of the SuperPlant Pre-filled Plate for Plant DNA Extraction (PF16Y)**.
7. Place the Magnetic Rod Tip (LA1118B) onto the machine.

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

NOTE: 16 samples can be processed in a single SuperPlant Pre-filled Plate for Plant DNA Extraction (PF16Y).

8. Close the door and Click on the **RUN** option on the home screen.
9. After the run is complete discard the Magnetic Rod Tip (LA1118B). Remove the SuperPlant Pre-filled Plate for Plant DNA Extraction (PF16Y) from the position. Dispense the eluted nucleic acid from column 6 and column 12 to a new HiPer® Lock Microcentrifuge Tube, 2.0ml (MBLA1017) (not provided). The eluate contains pure nucleic acid.

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 eluate contains pure DNA. For short-term storage (24-48 hrs.) of the DNA, 2-8°C is recommended. For long-term storage, recommended to be stored at -20°C or lower temperature (-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 Real- Time PCR.

Quality Control

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

Safety Information

The HiPurA® SuperPlant Pre- filled Plates for Plant DNA Extraction 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 of 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
	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		

Identification No.: PIMB571MPF16

Rev.No.:02

Date of Issue: 2026-01

Disclaimer :

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