

## MB507PC16 HiPurA® Pre- filled Cartridges for Plant DNA Purification

### Kit Contents

Product Code	Reagents provided	MB507PC16
		48 PR
PF160	Pre-filled Cartridges for Plant DNA Purification	48 nos
LA1118B	Magnetic Rod Tip	24 nos
DS0016	Lysis Buffer (PL)	20 ml
DS0054	Additive-I	0.14g
DS0017	Precipitation Buffer (PS)	6.5 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	6.5 ml
DS0003	RNase A (20mg/ml)	1 ml
DS1005A	Magnetic Beads	1 ml
DSCA02	HiShredder (in DBCA016 Collection tube)	48 No.

### Intended Use

Recommended for isolation of DNA from plant samples.

### Introduction

HiPurA® Pre- filled Cartridges for Plant DNA Purification provides the fastest and easiest way to purify DNA for reliable use in amplification technologies. HiPurA® Pre- filled Cartridges for Plant DNA Purification 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® Pre- filled Cartridges for Plant DNA Purification

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 as it is 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 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.

### HiShredder (DSCA02)

For the plant genomic 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.

## 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® Pre- filled Cartridges for Plant 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 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 ≈13,000 rpm [TabSpin® 012 (LA1090)]
- Ethanol (96-100%)
- 95°C and 65°C water bath or heating block
- Molecular Biology Grade Water (Product Code: ML064)
- Insta NX® Mag16 (Product Code: LA1096)
- Insta NX® Mag32 (LA1096)
- Cartridge Holder (LA1118CH)
- Vortex
- Polypropylene sealing film (Product Code: PR21)
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)
- Ice

## General Preparation Instructions

1. Preheat a water bath or heating block to 95°C or 65°C as required.
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. Prechill the mortar and pestle at -20°C.
5. Dissolve 2.6 mg of Additive-I in 400µl of Lysis Buffer (PL) and heat the solution to 95°C before use  
**(Only for Hard tissues).**

## 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**

#### For leaves/ flowers/ fruits/ stem

Collect plant tissue in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage.

#### For roots

Remove excess soil and collect plant tissue in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage.

It is preferable to use young plant parts especially leaves, needles (in case of pine, fir etc), since they contain more cells per weight and therefore result in higher yields. Also, young leaves and needles contain less polysaccharides and polyphenolics and are therefore easier to handle.

### **Types of Specimen**

Samples: leaves, flowers, fruits, stem and roots

### **Sample Preparation (Manual Disruption with mortar and pestle) with liquid Nitrogen**

**For Leaf Sample:** Finely cut the leaf material before grinding. Midrib should be removed before cutting the leaves as midrib is a major source of carbohydrate contamination. Weigh 100-200mg (depending upon species) 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.

**For Seeds:** Grind about 100mg of soaked seeds 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 (Manual Disruption with mortar and pestle) without liquid Nitrogen**

**For Leaf Sample:** Finely cut the leaf material before grinding. Midrib should be removed before cutting the leaves as midrib is a major source of carbohydrate contamination. Weigh 100-200mg (depending upon species) of the finely cut plant material and grind properly using a mortar and pestle in 400µl of Lysis Buffer (PL) (DS0016). Proceed immediately to the DNA **isolation protocol with step 2.**

**For Seeds:** Grind about 100mg of soaked seeds in in 400µl of Lysis Buffer (PL) (DS0016). Proceed immediately to the DNA isolation protocol.

**NOTE:** Delay in DNA isolation after sample preparation will result in DNA degradation and yield loss.

## A. Protocol for Hard and Soft tissues

### For Hard tissues

1. To the ground material (with liquid nitrogen), immediately add 400  $\mu$ l of Lysis Buffer (PL) (DS0016) containing Additive-I (DS0054) (preheated to 95°C) and mix thoroughly. [Do not grind the plant material after the addition of Lysis Buffer (PL), as it will cause shearing of DNA].

**NOTE:** For the preparation of Lysis Buffer (PL) refer General Preparation Instructions.

2. Transfer the mixture to a capped 2.0 ml collection tube (not provided). Vortex vigorously.
3. Incubate the mixture for 10 minutes at 95°C, mix the contents 2-3 times by inverting the tube.
4. Add 130  $\mu$ l of Precipitation Buffer (PS) (DS0017) to the lysate, mix and incubate for 5 minutes on ice.
5. **Load sample in HiShredder (DSCA02)**  
Add the entire sample to the HiShredder placed in a 2.0 ml collection tube (uncapped) and centrifuge for 5 minutes at a maximum speed ( $\approx$ 13,000 rpm) at room temperature (15-25°C). Transfer the flow-through fraction to a 2.0 ml collection tube (not provided) without disturbing the cell debris pellet.
6. Add 20  $\mu$ l of RNase A Solution (20 mg/ml) (DS0003) to the above tube and incubate at room temperature (15-25°C) for 10 minutes. **This will be your pre- processed sample.**

### For Soft tissues

1. To the ground material (with liquid nitrogen), immediately add 400  $\mu$ l of Lysis Buffer (PL) (DS0016) and mix thoroughly. [Do not grind the plant material after the addition of Lysis Buffer (PL), as it will cause shearing of DNA].
2. Transfer the mixture to a capped 2.0 ml collection tube (not provided). Vortex vigorously.
3. Add 20  $\mu$ l of RNase A Solution (20 mg/ml) (DS0003) to the above tube and incubate at room temperature (15-25°C) for 10 minutes.
4. Incubate the mixture for 10 minutes at 65°C, mix the contents 2-3 times by inverting the tube.
5. Add 130  $\mu$ l of Precipitation Buffer (PS) (DS0017) to the lysate, mix and incubate for 5 minutes on ice.
6. **Load sample in HiShredder (DSCA02)**  
Add the entire sample to the HiShredder placed in a 2.0 ml collection tube (uncapped) and centrifuge for 5 minutes at a maximum speed ( $\approx$ 13,000 rpm) at room temperature (15-25°C). Transfer the flow-through fraction to a 2.0 ml collection tube (not provided) without disturbing the cell debris pellet.  
**This will be your pre- processed sample.**

### **Set up processing Cartridges as follows:**

1. Switch on the UV light for 10 minutes prior to use.
2. Select “**MB50716**” program. Open the door of the Insta NX<sup>®</sup> Mag16 machine.
3. Remove the seal from the Pre-filled Cartridges for Plant DNA purification (PF16O).

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

4. Add 100µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040) into the 6<sup>th</sup> well of the Pre-filled Cartridges for Plant DNA purification (PF160).
5. Add 450µl pre- processed sample in the 1<sup>st</sup> well of the Pre-filled Cartridges for Plant DNA purification (PF160).
6. Add 20 µl Magnetic Beads (DS1005A) in the 1<sup>st</sup> well of the Pre-filled Cartridges for Plant DNA purification (PF160).
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 Pre-filled Cartridges for Plant DNA purification (PF160).**

8. Close the door and Click on the **RUN** option on the home screen.
9. After the run is complete, remove Cartridge Holder (LA1118CH) & cartridge from the position. Discard the Magnetic Rod Tip (LA1118B). Dispense the eluted DNA from well 6 to a new HiPer® Lock Microcentrifuge Tube, 2.0ml (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.

OR

Take out eluate in new collection tube and centrifuge at higher speed for around 1 min to pellet down the traces of Magnetic beads present in the eluate.

**\*NOTE: If you process less than 4 samples at a time please order LA1118B- Magnetic Rod Tip (Pack size- LA1118B-4no/ LA1118B-40no).**

**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® Pre- filled Cartridges for Plant DNA Purification is tested against predetermined specifications to ensure consistent product quality.

**Safety Information**

The HiPurA® Pre- filled Cartridges for Plant 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 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](mailto:mb@himedialabs.com).

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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		

Identification No.: PIMB507PC16

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### Disclaimer :

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