

MB507MPF16

HiPurA® Pre- filled Plates for Plant DNA Purification

Kit Contents

Product Code	Reagents provided	MB507MPF16
		96 PR
PF16Q	Pre-filled Plates for Plant DNA Purification	6 no
LA1118B	Magnetic Rod Tip	12 no
DS0016	Lysis Buffer (PL)	39 ml
DS0054	Additive-I	0.25 g
DS0017	Precipitation Buffer (PS)	12.5 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	12 ml
DS0003	RNase A Solution (20mg/ml)	2 ml
DS1005A	Magnetic Beads	2 ml
DSCA02	HiShredder (in DBCA016 Collection tube)	96 No.

Intended Use

Recommended for isolation of DNA from plant samples.

Introduction

HiPurA® Pre- filled Plates for Plant 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 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 Plates 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 plate.

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 Plates for Plant DNA Purification can be stored at room temperature (15-25°C) for up to 2 year 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
- 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: LA1118)
- Vortex
- Polypropylene sealing film (Product Code: PR21)
- Microcentrifuge Tube-C, 2.0ml (Product Code: PW147)
- 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 μ 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 μ 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 μ 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 μ 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 μ 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 μ 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 plates 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[®] Mag16 machine.
3. Remove the seal from the Pre-filled Plate for Plant DNA purification (PF16Q).

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] into **6th and 12th column of the Pre-filled plates for Plant DNA purification (PF16Q).**
5. Add **450µl pre- processed sample** in the **1st and the 7th column of the Pre-filled plate for Plant DNA Purification (PF16Q).**
6. **Add 20 µl Magnetic Beads (DS1005A) in the 1st and the 7th column of the Pre-filled Plate for Plant DNA Purification (PF16Q).**
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 Plate for Plant DNA Purification (PF16Q).

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 Pre-filled Plates for Plant DNA Purification (PF16Q) from the position. Dispense the eluted nucleic acid from column 6 and column 12 to a new HiPer[®] Lock Microcentrifuge Tube, 2.0ml (MBLA017) (not provided). The eluate contains pure nucleic acid.

NOTE: A small amount of magnetic beads may be observed in the final eluate at the bottom of the plate. Avoid transferring these magnetic beads to your PCR reaction mixture.

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 Plates for Plant DNA Purification is tested against predetermined specifications to ensure consistent product quality.

Please refer disclaimer Overleaf.

Safety Information

The HiPurA® Pre- filled Plates 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.

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 :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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