MB520 HiPurA® Plant Genomic DNA Maxiprep Purification Kit

Kit Contents

Product Code	Reagents provided	MB520	
		10 Preps	25 Preps
DS0016	Lysis Buffer (PL)	60 ml	150 ml
DS0054	Additive-I	390 mg	975 mg
DS0017	Precipitation Buffer (PS)	19 ml	47.5 ml
DS0019	Wash Solution Concentrate (WSP)	30 ml	75 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	20 ml	50 ml
DS0003	RNase A Solution (20 mg/ml)	0.6 ml	1.5 ml
DSCC01	HiShredder Maxi (in PW143 Collection Tube)	10 nos	25 nos
PW143	Collection Tubes (50 ml conical)	20 nos	50 nos

Intended Use

Recommended for isolation of DNA from Plant samples.

Introduction

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

HiPurA® Plant Genomic DNA Maxiprep Purification Kit

This kit simplifies isolation of DNA from fresh plant material. The procedure is optimized for a maximum of 1 g of wet-weight starting material. The sample (fresh) is cut and ground in liquid nitrogen and then mixed with Lysis Buffer (PL). Protein precipitation is followed by removal of other contaminants using HiShredder Maxi. The flow-through fraction is then mixed with isopropanol to precipitate DNA. Washing steps to remove trace contaminants will follow this step. High quality DNA is dissolved in the Elution Buffer (ET) provided in the kit. Typical yield is up to 50-250 µg/gram of leaf material.

HiShredder Maxi (DSCC01)

For the HiMedia Plant Genomic DNA isolation procedure, contaminants such as cell debris, salt precipitates are removed by centrifugation through a HiShredder Maxi. The HiShredder Maxi 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. Dissolving DNA in 1 ml of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. The DNA obtained ranges in size upto 50 kb (predominant fragment size 20-30 kb), and is suitable for direct use in PCR, restriction digestion, and

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WHO GMP CERTIFIED
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Southern blotting applications.

Concentration, yield and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and 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® Plant Genomic DNA Maxiprep 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

- Mortar and Pestle
- Liquid nitrogen
- Tabletop Centrifuge (with rotor for 50.0 ml tubes). **Swinging bucket rotor is recommended over fixed angle rotor.**
- Water bath or heating block at 65°C or 95°C
- Isopropanol (2-Propanol) (Product Code: MB063)
- Ethanol (96-100%)
- Molecular Biology Grade Water (Product code: ML024)

Storage

Store the HiPurA® Plant Genomic DNA Maxiprep Purification Kit between 15-25°C except certain components as specified on each label. Under recommended condition kit is stable for 18 months.

General Preparation Instructions

1. Preheat a water bath or heating block to 65°C or 95°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 centrifuge tubes and tips are used for the procedure.
4. **Dilute Wash Solution Concentrate (WSP) (DS0019) as follows:**

Number of Preps	Wash Solution Concentrate (WSP)	Ethanol (96-100 %)
10	30 ml	70 ml
25	75 ml	175 ml

5. Prechill the mortar and pestle at -20°C.
6. Dissolve 32.5 mg of Additive-I in 5 ml of Lysis Buffer (PL) and heat the solution to 95°C 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).

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: We recommend our users to follow different protocols for soft and hard tissues.

NOTE: 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.

Specimen Collection and Handling

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.

Types of Specimen

Samples: leaves, flowers, fruits, stem and roots

Sample Preparation (Manual Disruption with mortar and pestle)

Sample types:

1) Fresh plant material - can be disrupted in Lysis Buffer without liquid nitrogen, however grinding to a fine powder in liquid nitrogen and then immediately adding the Lysis Buffer gives better results.

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 1 g 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.

Protocol

A. <u>For Soft tissues</u>

1. To the ground material, immediately add 5 ml of Lysis Buffer (PL) (DS0016) and mix thoroughly (Do not grind the plant material after the addition of PL, as it will cause shearing of DNA). Add 50 µl of RNase A (20 mg/ml) to the sample.

2. Transfer the mixture to a 50 ml collection tube. Vortex vigorously.

3. Incubate the mixture for 10 minutes at 65°C, mix the contents 2-3 times by inverting the tube **(Continue with Step 4)**.

B. <u>For Hard tissues</u>

1. To the ground material, immediately add 5 ml 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 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 50 ml collection tube. Vortex vigorously.

3. Incubate the mixture for 10 minutes at 95°C, mix the contents 2-3 times by inverting the tube **(Continue with Step 4)**.

4. Add 1.8 ml of Precipitation Buffer (PS) (DS0017) to the lysate, mix and incubate for 10 minutes on ice.

5. **Load sample in HiShredder (DSCA01)**
   Add the entire sample to the HiShredder placed in a 50 ml collection tube and centrifuge for 5 minutes at 3,000-5,000 x g (≈5000 rpm).

6. Transfer the flow-through without disturbing the cell debris pellet into a new 50 ml collection tube.

7. Add 50 µl of RNase A (20 mg/ml) (DS0003) (**Skip this step incase using soft tissues**). Mix the sample gently by inversion and incubate for 30 minutes at room temperature (15-25°C).

   NOTE: Skip this step incase using soft tissues.

8. Add 6 ml of isopropanol (2-Propanol). Mix the sample gently by inversion until a white fluffy DNA precipitate appears (it should appear within 1 minute of addition of isopropanol).

9. Centrifuge the lysate for 5 minutes at 3,000-5,000 x g (≈5000 rpm) to precipitate the DNA. Discard the supernatant.

10. **Wash**
    (Prepare the Wash Solution as indicated in General Preparation Instructions)
    To the pellet, add 8 ml of diluted Wash Solution (WSP) (DS0019). Mix the sample gently by inversion and incubate for 20 minutes at room temperature (15-25°C). Centrifuge for 5 minutes at 3,000-5,000 x g (≈5000 rpm). Discard the supernatant (Wash Solution).

11. Air dry the pellet for 5 minutes to remove the traces of ethanol present in the Wash Solution.

12. Dissolve the pellet in 1 ml of the Elution Buffer (ET) (DS0040).

NOTE: Storing DNA in water can cause acid hydrolysis.

Storage of the eluate with purified DNA: The eluate contains pure genomic DNA. For short-term storage (24-48 hours) 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 (ET) 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® Plant Genomic DNA Maxiprep Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Yield	DNA Purity
Plant leaf sample (1 g)	50-250 µg of DNA	1.6-1.9

References

1. Sambrook, J., *et al.* Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989).
2. Birren, B. and Lai, E. Pulsed Field Gel Electrophoresis: A practical guide (Academic Press, San Diego, CA, 1993).

Troubleshooting guide

Sr. No.	Problem	Possible Cause	Solution
1.	Lower yields of DNA	Insufficient disruption of the plant tissue	Ensure that the plant material is disrupted in sufficient amounts of liquid nitrogen. It is very important that the disrupted tissue sample should not thaw before addition of Lysis Buffer.
		Insufficient lysis	The amount of the starting material can be reduced or the amount of buffers PL and PS can be increased.
		Insufficient centrifugation	The g-force and the centrifugation time can be increased.

2.	Shearing of DNA	Precipitation of Lysis Buffer (PL)	Examine the solution for any kind of precipitation; if the solution forms a precipitate, warm at 55-65°C until the precipitate dissolves completely, allow it to cool to room temperature (15-25°C) before use.
3.	Purity of the DNA is lower than expected.	DNA is contaminated with RNA	50 µl of RNase A (20 mg/ml) can be added to the lysate and incubate as mentioned in the protocol.
4.	Carbohydrate contamination in the sample.	Grinding of the midrib along with the leaf material	Remove the midrib from the leaf before grinding. Removal of the midrib is not important in case of very young leaves.
5.	DNA appears degraded (as a smear running down the gel).	DNA appears fragmented or broken	DNA being a large molecule can be broken by shear forces if treated vigorously. Therefore, mix the samples gently, never vortex the DNA. To minimize shearing, always use a wide bore pipette tip for mixing.
6.	Difficulty to dissolve DNA in Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	This is due to over-drying of DNA pellet	The DNA should not be allowed to over-dry at any stage during the preparation as it hinders the solubilization in Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]

Safety Information

The HiPurA® Plant Genomic DNA Maxiprep Purification Kit is for laboratory use only, not for drug, household or other uses. Precipitation Buffer (PS) contains acetic acid, which is an irritant. 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.

Symbols

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

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