MB571 HiPurA® SuperPlant DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB571		
		20 Preps	50 Preps	250 Preps
DS0200	SuperPlant Extraction Buffer	22 ml	55 ml	275 ml
DS0070	Additive-II	2 ml	5 ml	25 ml
DS0071	Additive-III	0.5 g	1.25 g	6.25 g
DS0003	RNase A Solution (20 mg/ml)	500 µl	1.25 ml	6.25 ml
DS0019	Wash Solution Concentrate (WSP)	12 ml	30 ml	150 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	2.4 ml	6 ml	30 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos	50nos	250 nos
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
PW1139	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

Introduction

DNA extraction from plant tissues, unlike DNA isolation from mammalian tissues, remains difficult due to the presence of a rigid cell wall surrounding the plant cells. This method can be used both on freeze dried leaves and on fresh leaves. The scale of extraction is dependent on the amount of starting material, for e.g. 200 mg of material requires 900 µl of SuperPlant Extraction Buffer and yields 10-50 µg of DNA.

HiPurA® SuperPlant DNA Purification Kit

This kit simplifies isolation of DNA from fresh plant material with spin column procedure. The procedure is optimized for a maximum of 200 mg of wet-weight of the starting material. The sample (fresh) is cut and ground in liquid nitrogen along with SuperPlant Extraction Buffer. SuperPlant Extraction Buffer contains CTAB (Cetyltrimethylammonium bromide), a detergent used to break open plant cells and solubilize the contents. Chlorophyll and some denatured proteins are removed from green plant tissue in an organic chloroform- isoamylalcohol step, and the organic phase is separated by centrifugation. Since the extract contains DNA and RNA, RNA can be removed by the addition of RNase A. The flow-through fraction is then mixed with a solution that enhances the binding of DNA to the column. The solution is then passed through HiElute Miniprep Spin Column (Capped) that is followed by washing steps to remove trace contaminants. High quality DNA is eluted in the Elution Buffer (ET) provided in the kit.

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube] [DBCA03]

HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube] is based on the advanced silica binding principle presented in a microspin format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced gel membrane and the speed plus versatility of spin column technology to yield high quantity of DNA. The use of spin column facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. This column eliminates the need for alcohol precipitation, expensive resins, and harmful organic compounds such as phenol and chloroform, otherwise employed in traditional DNA isolation techniques. DNA binds specifically to the advanced silica-gel membrane while contaminants pass through. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps, leaving pure nucleic acid to be eluted in the buffer provided with the kit. The purified DNA is upto 20 - 30 kb in length and can be used for further downstream applications.

Elution

The yield of genomic DNA depends on the sample type and the number of cells in the sample. Elution with 100µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 100µl will increase the final DNA concentration, but will reduce the overall DNA yield. The eluted DNA ranges in size upto 20-30 kb and is suitable for direct use in PCR, restriction endonuclease digestion, Southern blotting applications etc.

Concentration, yield and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the 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® SuperPlant DNA 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.

Key advantage: This method can be used to process large number of samples on a daily basis. Very high DNA yield is obtained (upto 50 µg of DNA).

Materials needed but not provided:

- Chloroform: Isoamylalcohol (24:1)(Product Code: MB115)
- Ethanol (96-100%)
- Mortar and pestle
- Liquid nitrogen
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- 65°C water bath or heating block

Storage

Store the HiPurA® SuperPlant DNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 1 year.

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 a 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.

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 µl of Additive-II and 18 mg of Additive-III in 900 µl of SuperPlant Extraction Buffer. Preheat the solution to 65°C.

3. **Dilute Wash Solution Concentrate (WSP) (DS0019) as follows:**

Number of Preps	Wash Solution Concentrate (WSP)	Ethanol (96-100 %)
20	12 ml	28 ml
50	30 ml	70 ml
250	150 ml	350 ml

DNA Isolation Protocol

Sample Preparation

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.

Protocol

NOTE: Ensure that Additive-II and Additive-III are added to SuperPlant Extraction Buffer (DS0200) as mentioned in General Preparation Instructions.

1. To 200 mg of the ground material add 900 µl of SuperPlant Extraction Buffer (DS0200) (preheated to 65°C) (Refer General Preparation Instructions) and transfer the sample to a capped 2.0 ml collection tube. 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) 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 fresh 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).

6. Add equal volume of Ethanol (96-100%) to the lysate obtained from the above step and mix by pipetting.

7. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Add 650 µl of the lysate, including any precipitate, which may have formed, to the column placed in a uncapped 2.0ml collection tube. Centrifuge for 1 minute at 6000 x g (≈8000 rpm). Discard the flow-through.

8. Repeat the above step with the remaining sample. Discard the flow-through liquid and reuse the 2.0 ml collection tube (uncapped).

9. **Wash**
   (Prepare the diluted Wash Solution (WSP) (DS0019) as indicated in General Preparation Instructions)
   Add 500 µl of diluted Wash Solution (WSP) and centrifuge for 1 minute at 6000 x g (≈8000 rpm).

   NOTE: Discard the flow-through and reuse the 2.0 ml collection tube (uncapped).

10. Add another 500 µl of diluted Wash Solution (WSP) to the column and centrifuge for 2 minutes at a maximum speed (≈14,000 rpm). Discard the flow-through and reuse the same collection tube.

11. Centrifuge the tube with column for an additional 2 minutes at a maximum speed (≈14,000 rpm) to dry the membrane.

12. **DNA Elution**
    Place the column in a new 2.0ml collection tube (uncapped) and pipette 100 µl of the Elution Buffer (ET) (DS0040) directly onto the column without spilling to the sides. Incubate for 5 minutes at room temperature (15-25°C). Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute to elute the DNA. Transfer the eluate to a new capped 2.0ml collection tube for DNA storage.

    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.

Precautions

Read the procedure carefully before starting the experiment.

Performance and Evaluation

Each lot of HiMedia's HiPurA® SuperPlant DNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Yield	DNA Purity
Mango leaf	10-50 µg of DNA	1.6-1.9

Troubleshooting Guide

Sr. No.	Problem	Probable Cause	Solution
1.	Carbohydrate contamination in the sample	Grinding of the midrib along with the leaf material	Remove the midrib from the leaf before grinding as these plant species have prominent midribs. Removal of the midrib is not important in case of very young leaves.
2.	DNA appears degraded (as a smear running down the gel)	The plant material for freeze-drying is not immediately frozen	When harvesting plant material for freeze drying, ensure that the tissue is immediately frozen, as this reduces DNA degradation.
		DNA appears fragmented or broken	DNA being a large molecule can be broken by shear forces. Therefore, mix the samples gently. To minimize shearing, always use a wide bore pipette tip for mixing.
3.	Difficulty to dissolve DNA in Elution Buffer (ET)	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 resuspension and solubilization in Elution Buffer (ET). Rehydrate the DNA by incubating at 65°C for 1 hour in Elution Buffer (ET).

Safety Information

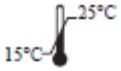
HiPurA® SuperPlant DNA Purification Kit is for laboratory use only; not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Avoid contact with skin, and use eye protection. In case of contact, wash with large amount of water. Seek medical attention. 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.



Storage temperature

Do not use if package is damaged

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