MB533  
HiPurA® Banana DNA Isolation Kit

**Kit Contents**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Reagents provided</th>
<th>MB533</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS0069</td>
<td>Banana Extraction Buffer</td>
<td>50 ml</td>
</tr>
<tr>
<td>DS0070</td>
<td>Additive-II</td>
<td>5 ml</td>
</tr>
<tr>
<td>DS0071</td>
<td>Additive-III</td>
<td>1 g</td>
</tr>
<tr>
<td>DS0003</td>
<td>RNase A Solution (20 mg/ml)</td>
<td>1.25 ml</td>
</tr>
<tr>
<td>DS0072</td>
<td>Banana Wash Buffer Concentrate (WB1)</td>
<td>60 ml</td>
</tr>
<tr>
<td>DS0073</td>
<td>Precipitation Buffer (BS)</td>
<td>3 ml</td>
</tr>
<tr>
<td>DS0005</td>
<td>Elution Buffer (EB) [10mM Tris-Cl, 1.0mM EDTA, pH 8.5]</td>
<td>10 ml</td>
</tr>
<tr>
<td>PW1139</td>
<td>Collection Tubes, Polypropylene (2.0 mL)</td>
<td>100 nos.</td>
</tr>
</tbody>
</table>

**Intended Use**

Recommended for isolation of DNA from Banana leaf samples.

**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 Extraction Buffer and yields 10-50 µg of DNA.

**Principle**

Banana 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 DNA is precipitated and washed in organic solvents before re-dissolving in aqueous solution.

**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:**

- 2-mercaptoethanol (ß-ME) (Product Code: MB041)
- Chloroform: Isoamylalcohol (24:1) (Product Code: MB115)
- 2-Propanol (Isopropanol) (Product Code: MB063)
- Mortar and pestle
- Liquid nitrogen
- Tabletop Microcentrifuge (with rotor for 2.0mL tubes)
- 65°C water bath or heating block

The information contained herein is believed to be accurate and complete. However, no warranty or guarantee whatsoever is made or is to be implied with respect to such information or with respect to any product, method or apparatus referred to herein.

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Web: www.himedialabs.com

Storage

Store the HiPurA® Banana DNA Isolation 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 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).

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 (15-25°C) and are given in g, the correct rpm can be calculated using the formula:

\[
RPM = \sqrt{\frac{RCF}{1.118 \times 10^{-5} r}}
\]

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 with liquid nitrogen. Midrib should be removed from the material before grinding, as midrib is a major source of carbohydrate contamination.

2. **Banana Extraction Buffer:** Immediately prior to use, add 90 µl of Additive-II, 18 mg of Additive-III and 18 µl of β-mercaptoethanol in 900 µl of Banana Extraction Buffer. Preheat the solution to 65°C.

3. **Dilute Banana Wash Buffer Concentrate (WB1) (DS0072) as follows:**

<table>
<thead>
<tr>
<th>Number of Preps</th>
<th>Banana Wash Buffer Concentrate (WB1)</th>
<th>Ethanol (96-100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>60 ml</td>
<td>140 ml</td>
</tr>
<tr>
<td>250</td>
<td>300 ml</td>
<td>700 ml</td>
</tr>
</tbody>
</table>

Banana Leaf DNA: Using MB533- HiPurA® Banana DNA Isolation Kit

Specimen Collection and Handling

For leaves
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

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, Additive-III and β-mercaptoethanol are added to Banana Extraction Buffer as mentioned in General Preparation Instructions.

1. To 200 mg of the ground material add 900 µl of Banana Extraction Buffer (DS0069) (preheated to 65°C) (Refer General Preparation Instructions) and transfer the sample to a 2.0 mL collection tube (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) and mix gently by inversion for 5 minutes.

4. Centrifuge the samples at 10,000 x g [=13,000 rpm] for 10 minutes at room temperature (15-25°C).

5. Transfer the top aqueous layer (containing DNA) into a new tube and add 1 mL of Isopropanol and gently mix by inversion for 5 minutes.

NOTE: After the addition of isopropanol, DNA will start to precipitate within 1-2 minutes.

6. Centrifuge the samples at 10,000 x g [=13,000 rpm] for 10 minutes at room temperature (15-25°C). Discard the supernatant.
7. Add 1 mL of diluted Banana Wash Buffer (WB1) (DS0072) and resuspend the DNA pellet by pipetting up and down. Centrifuge at 10,000 x g [=13,000 rpm] for 10 minutes at room temperature (15-25°C). Discard the supernatant.

**NOTE:** Prepare Wash Buffer as indicated in General Preparation Instructions.

8. Repeat the above washing step one more time.

9. Air dry the pellet for 10-15 minutes and resuspend in 300 µl of Elution Buffer (EB) (DS0005).

10. Add 20 µl of RNase A Solution (20 mg/mL) (DS0003) and incubate for 30 minutes at room temperature (15-25°C).

11. Add 30 µl of Precipitation Buffer (BS) (DS0073) and 210 µl of isopropanol. Mix by inversion for 5 minutes.

12. Centrifuge at 10,000 x g [=13,000 rpm] for 15 minutes at 4°C. Discard the supernatant.

13. Add 1.5 mL of diluted Banana Wash Buffer (WB1) (DS0072) and resuspend the pellet by pipetting up and down. Centrifuge at 10,000 x g [=13,000 rpm] for 10 minutes at room temperature (15-25°C).

14. Discard the supernatant and air dry the pellet until the residual ethanol has completely evaporated.

15. Resuspend the DNA pellet in 100 µl of Elution Buffer (EB) (DS0005).

**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® Banana DNA Isolation Kit is tested against predetermined specifications to ensure consistent product quality.

**Quality Control**

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>DNA Yield</th>
<th>DNA Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana leaf sample</td>
<td>5-40 µg of DNA</td>
<td>1.6-1.9</td>
</tr>
</tbody>
</table>

**Troubleshooting Guide**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Problem</th>
<th>Probable Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carbohydrate contamination in the sample</td>
<td>Grinding of the midrib along with the leaf material</td>
<td>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.</td>
</tr>
<tr>
<td>2.</td>
<td>DNA appears degraded (as a smear running down the gel)</td>
<td>The plant material for freeze-drying is not immediately frozen</td>
<td>When harvesting plant material for freeze drying, ensure that the tissue is immediately frozen, as this reduces DNA degradation.</td>
</tr>
<tr>
<td>DNA appears fragmented or broken</td>
<td>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.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Difficulty to dissolve DNA in Elution Buffer (EB)</td>
<td>This is due to over-drying of DNA pellet</td>
<td>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 (EB). Rehydrate the DNA by incubating at 65°C for 1 hour in Elution Buffer (EB).</td>
</tr>
</tbody>
</table>

### Safety Information

HiPurA® Banana DNA Isolation 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

HiMedia Laboratories Pvt. Limited,
23 Vadhani Industrial Estate,
LBS Marg, Mumbai-86, MS, India

Disclaimer:
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