# MB567 HiPurA® Spice DNA Purification Kit

## Kit Contents

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Reagents provided</th>
<th>MB567</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS0152</td>
<td>Spice Extraction Buffer</td>
<td>55 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>1.25 g</td>
</tr>
<tr>
<td>DS0003</td>
<td>RNase A Solution (20 mg/ml)</td>
<td>1.25 ml</td>
</tr>
<tr>
<td>DS0019</td>
<td>Wash Solution Concentrate (WSP)</td>
<td>24 ml</td>
</tr>
<tr>
<td>DS0040</td>
<td>Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]</td>
<td>6 ml</td>
</tr>
<tr>
<td>DBCA03</td>
<td>HiElute Miniprep Spin Column (Capped)</td>
<td>50 nos</td>
</tr>
<tr>
<td>DBCA016</td>
<td>Collection Tubes (uncapped), Polypropylene (2.0 ml)</td>
<td>50 nos</td>
</tr>
<tr>
<td>PW1139</td>
<td>Collection Tubes, Polypropylene (2.0 ml)</td>
<td>100 nos</td>
</tr>
</tbody>
</table>

## Introduction

Different types of spices contain high levels of polysaccharides, polyphenol compounds and secondary metabolites. Polysaccharides and polyphenols are not completely removed during classical extraction protocols; they remain as contaminants in the final DNA preparations. Polysaccharides make DNA viscous, glue-like and non-amplifiable in the PCR reaction by inhibiting Taq enzyme activity and also interfere with accurate activity of restriction enzymes.

**HiPurA® Spice DNA Purification Kit**

This kit simplifies purification of DNA from spice sample with spin column procedure. The procedure is optimized for a maximum of 200 mg of dry-weight of the starting material. The sample is ground in liquid nitrogen along with Spice Extraction Buffer. Spice Extraction Buffer contains CTAB (Cetyltrimethylammonium bromide), a detergent used to break open cells and solubilize the contents. Some denatured proteins are removed from tissues in an organic chloroform-isooamylalcohol 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. The yield of DNA is dependent on the amount of starting material.
HiElute Miniprep Spin Column (Capped) (DBCA03)

HiElute Miniprep Spin Column (Capped) 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 purification 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 up to 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 100 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 100 µl will increase the final DNA concentration, but will reduce the overall DNA yield. The eluted DNA ranges in size up to 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 $A_{260}/A_{320}$ 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® Spice 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.

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

HiPurA® Spice DNA Purification Kit can be stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance.

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.

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:

$$RPM = \sqrt{RCF/1.118 \times 10^{-5} \times r}$$

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 spice sample can be done using mortar and pestle.
2. **Spice Extraction Buffer**: Immediately prior to use, add 90 µl of Additive-II and 18 mg of Additive-III in 900 µl of Spice Extraction Buffer. Preheat the solution to 65°C.
3. **Dilute Wash Solution Concentrate (WSP) (DS0019) as follows:**
   
<table>
<thead>
<tr>
<th>Number of Preps</th>
<th>Wash Solution Concentrate (WSP)</th>
<th>Ethanol (96-100 %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>24 ml</td>
<td>56 ml</td>
</tr>
</tbody>
</table>

DNA Purification Protocol

Sample Preparation
Weigh 200 mg of spice sample 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 purification protocol.

Protocol

**NOTE:** Ensure that Additive-II and Additive-III are added to Spice Extraction Buffer as mentioned in General Preparation Instructions.

1. To 200 mg of the ground material add 900 µl of Spice Extraction Buffer (DS0152) (preheated to 65°C) (Refer General Preparation Instructions) and transfer the sample to a Collection tube, Polypropylene (2.0 ml) (provided). Mix by vortexing.
2. Incubate the samples for 60-90 minutes at 65°C with occasional inversion.
3. Add 1 ml of Chloroform: Isoamyl alcohol (24:1) and mix gently by inversion for 5 minutes.
4. Centrifuge the samples at 13,000 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 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) (DBC093)
   Add 650 µl of the lysate, including any precipitate, which may have formed, to the column
   placed in a Collection tube (Uncapped), Polypropylene (2.0 ml). 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 Collection tube (Uncapped), Polypropylene (2.0 ml).

9. Wash
   (Prepare the Wash Solution Concentrate (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). Discard the flow-through and reuse the Collection tube (Uncapped), Polypropylene
   (2.0 ml).

10. Add another 500 µl of diluted Wash Solution (WSP) to the column and centrifuge for 2
    minutes at a maximum speed (≈13,000 rpm). Discard the flow-through and reuse the
    same Collection tube (Uncapped), Polypropylene (2.0 ml).

11. Centrifuge the tube with column for an additional 2 minutes at a maximum speed
    (≈13,000 rpm) to dry the membrane.

12. DNA Elution
    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.

   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® Spice DNA Purification Kit is tested against predetermined
specifications to ensure consistent product quality.

Quality Control

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>DNA Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spice sample (200 mg)</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>DNA appears degraded (as a smear running down the gel)</td>
<td>The spice sample for freeze-drying is not immediately frozen</td>
<td>When harvesting spice sample for freeze drying, ensure that the tissue is immediately frozen, as this reduces DNA degradation.</td>
</tr>
</tbody>
</table>
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.

| 2. 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® Spice 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

15°C

Do not use if package is damaged

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