

## MB528

## HiPurA® Cotton DNA Isolation Kit

### Kit Contents

Product Code	Reagents provided	MB528	
		50 Preps	250 Preps
DS0048	Solution CD1	60 ml	300 ml
DS0049	Solution CD2	5 ml	25ml
DS0050	Solution CD3	25 ml	125 ml
DS0051	Solution CD4	7.5 ml	37.5 ml
DS0052	Solution CD5	7.5 ml	37.5 ml
MB086	Proteinase K	6 mg	30 mg
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	10 ml	50 ml

### Intended Use

Recommended for isolation of DNA from Cotton leaves.

### 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. Cotton leaves contain high levels of polyphenolic compounds that irreversibly interact with proteins and nucleic acids during DNA isolation. In order to overcome the problems associated with poor quality of DNA, this kit has been developed. The kit helps to extract good quality DNA which is compatible for downstream applications. This method can be used for both on Herbarium specimens and on fresh leaves. The scale of extraction is dependent on the amount of starting material. Typical yield from 100 mg of wet weight sample is 7-12 µg.

### Principle

Solution CD1 used in this kit contains polyvinyl pyrrolidone that binds the phenolic compounds. In the presence of PVP, phenolics adhere to DNA in solution and form a colored extract around DNA, which can be removed by the addition of Solution CD2 containing SDS. A high molar concentration of NaCl in Solution CD4 increases the solubility of polysaccharides in ethanol, effectively decreasing co-precipitation of the polysaccharides and DNA. Solution CD5, containing LiCl, for selective precipitation of large molecules of RNA thereby reducing the amount of RNA in final DNA concentration. Finally, pure DNA is eluted in Elution Buffer (ET). The concentration of DNA can be estimated by agarose gel electrophoresis.

### 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) or Molecular Biology Grade Water 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® Cotton DNA Isolation Kit is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

Concentration of DNA sample ( $\mu\text{g/ml}$ ) =  $50 \times A_{260} \times \text{dilution factor}$ .

#### **Materials needed but not provided:**

- Mortar and pestle
- Liquid Nitrogen
- Water bath or heating block at 55°C
- Tabletop Micro centrifuge (with rotor for 2.0 ml tubes)
- 2-Propanol (Isopropanol) (Product Code: MB063)
- RNase A Solution (20 mg/ml) (DSO003)
- Ethanol (96 –100%)
- Ethanol (70%)
- Molecular Biology Grade Water (ML024)
- Dithiothreitol (DTT (MB070)

#### **Storage**

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

#### **General Preparation Instructions**

1. Grinding of the plant material can be done using mortar and pestle. Midribs should be removed from the material before grinding, as midrib is a major source of carbohydrate contamination. Grind the weighed plant material using a mortar and pestle in liquid nitrogen to a fine powder. Transfer the tissue powder and liquid nitrogen to an appropriately sized tube and allow the liquid nitrogen to evaporate. **DO NOT ALLOW THE SAMPLE TO THAW.** (Keep the samples on ice if needed). Proceed immediately to the DNA isolation protocol.

**NOTE:** Delay in continuing DNA isolation after sample preparation will result in DNA degradation and yield loss.

2. Alternative method: Grind the weighed plant material using a mortar and pestle in liquid nitrogen to a fine powder. Upon evaporation of liquid nitrogen, immediately add Solution CD1 directly to the sample in the mortar and mix homogenously with the pestle. The mixture can then be scraped with a spatula into a tube. This ensures that there is no degradation of DNA.
3. Fresh plant material: Fresh plant material can be disrupted in Solution CD1 without liquid nitrogen. However, if you grind fresh plant material to a fine powder in liquid nitrogen followed by immediate addition of Solution CD1, it gives better results.
4. **Reconstitute Proteinase K (MB086)**  
The HiPurA® Cotton DNA Isolation Kit contains Proteinase K. Intensive research has shown that it is the optimal enzyme for use with the Extraction Buffer provided in the kit. It is completely free of DNase and RNase activity. The specific activity of Proteinase K is 33.5 units/mg dry weight.

Resuspend the Proteinase K (MB086) powder in Molecular Biology Grade Water (ML024) to obtain a 20 mg/ml stock solution.

Number of Preps	Proteinase K	Molecular Biology Grade Water
50	6 mg	0.3 ml
250	30 mg	1.5 ml

Proteinase K as supplied is stable at room temperature (15-25°C). However, once reconstituted store at -20°C as mentioned in storage instructions.

**NOTE:** The Proteinase K solution must be added to Solution CD1 prior to use.

Solution CD1: Immediately prior to use, add 10 µl of 1M Dithiothreitol (DTT) per ml of Solution CD1 and mix thoroughly.

Prechill Ethanol (96-100%) and Solution CD5 prior to use.

### 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. 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.

### Specimen Collection and Handling

#### For leaves

Collect cotton leaves 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

### Procedure

1. To 100 mg of the lyophilized ground tissue in a sterile collection tube, add 1 ml of Solution CD1 (DS0048) with Proteinase K.

**NOTE:** Prior to use, add 10 µl of 1M Dithiothreitol (DTT) to 1ml of Solution CD1 and mix thoroughly. To the mixture, add 5 µl of Proteinase K solution. Refer General Preparation Instructions for Proteinase K reconstitution.

2. Incubate the samples for 60 minutes, with occasional swirling at 55°C.
3. Add 75 µl of Solution CD2 (DS0049) to each tube. Mix the tube gently and incubate the samples for 60 minutes, with occasional swirling at 55°C.
4. Centrifuge for 10 minutes at 13,000 x g (≈14,000 rpm) at room temperature (15-25°C). Transfer the supernatant to a new tube.
5. Add 1/3 volumes of Solution CD3 (DS0050). Mix the tube gently and incubate for 30 minutes at -20°C.
6. Centrifuge for 10 minutes at 13,000 x g (≈14,000 rpm) at room temperature (15-25°C). Transfer the supernatant to a new tube.

7. Add 0.6 volumes of isopropanol. Mix the tube gently and incubate at -20°C overnight.
  8. Centrifuge for 10 minutes at 13,000 x g ( $\approx$ 14,000 rpm) at room temperature (15-25°C). Carefully discard the supernatant taking care that the pellet remains intact. Dissolve the pellet in 100  $\mu$ l Molecular Biology Grade Water(MLO24).
  9. Add 50  $\mu$ l of Solution CD4 (DS0051) and mix well. To that add 2 volumes of chilled ethanol (96-100%) and incubate for 30 minutes at -20°C.
  10. Centrifuge for 10 minutes at 13,000 x g ( $\approx$ 14,000 rpm) at room temperature (15-25°C). Discard the supernatant and dissolve the pellet in 100  $\mu$ l Molecular Biology Grade Water.
  11. To the dissolved pellet add 1/3 volumes of chilled Solution CD5 (DS0052) to precipitate RNA. Incubate for 60 minutes at -20°C.
  12. Centrifuge at 13,000 x g ( $\approx$ 14,000 rpm) for 15 minutes at room temperature (15-25°C). Carefully transfer the supernatant to a new tube without disturbing the pellet as the pellet contains RNA.
  13. To precipitate DNA, add 0.6 volumes of isopropanol to the supernatant and incubate for 60 minutes at -20°C.
  14. Centrifuge for 10 minutes at 13,000 x g ( $\approx$ 14,000 rpm) at room temperature (15-25°C). Carefully discard the supernatant.
  15. Wash the pellet by adding 500  $\mu$ l of 70% ethanol and centrifuge for 5 minutes at 13,000 x g ( $\approx$ 14,000 rpm) at room temperature (15-25°C).
- NOTE:** Discard the ethanol and briefly dry the pellet.
16. Dissolve the pellet in 50-100  $\mu$ l of Elution Buffer (ET) (DS0040).

### 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® Cotton DNA Isolation Kit is tested against predetermined specifications to ensure consistent product quality.

### Quality Control

Type of Sample	DNA Yield	DNA Purity
Cotton leaves	7-12 $\mu$ g	1.6-1.9

### References

1. Ribeiro, R.A., and Lovato, M.B. (2007) Comparative analysis of different DNA extraction protocols in fresh and herbarium specimens of the genus *Dalbergia*. Genet. Mol. Res. (6): 173 – 187.

## Troubleshooting Guide

Sr. No.	Problem	Probable Cause	Solution
1.	Purity of the DNA is lower than expected	DNA is contaminated with RNA	2.5 µl of RNase A (20 mg/ml) can be added to the eluted DNA and incubate for 15 minutes at 37°C.
2.	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.
3.	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 if treated vigorously. Therefore, mix the samples gently, never vortex the DNA. To minimize shearing always use a wide bore pipette tip for mixing.
4.	Difficulty to dissolve DNA in Elution Buffer	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.

## Safety Information

HiPurA® Cotton 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](mailto:mb@himedialabs.com).



Storage temperature



Do not use if package is damaged



HiMedia Laboratories Private Limited,  
Reg. Off: Plot No. C-40, Road No. 21Y,  
MIDC, Wagle Industrial Area, Thane,  
(West) 400604, Maharashtra, INDIA.  
Web: [www.himedialabs.com](http://www.himedialabs.com)



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HiMedia Laboratories Pvt. Ltd. Reg. office : Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane, (West) 400604, Maharashtra, INDIA.  
Customer Care No.: 00-91-22-6116 9797 Tel: 00-91-22-6147 1919, 6903 4800 Email: [techhelp@himedialabs.com](mailto:techhelp@himedialabs.com) Website: [www.himedialabs.com](http://www.himedialabs.com)