

MB502 HiPurA® Plant DNA Isolation Kit (CTAB Method)

Kit Contents

Product Code	Reagents provided	MB502
		50 Preps
DS0002	CTAB Extraction Buffer	500 ml
DS0003	RNase A Solution (20 mg/ml)	1.75 ml
DS0004	CTAB Wash Buffer Concentrate	120 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	100 ml
DS0029	CTAB Powder	5 grams

Intended Use

Recommended for isolation of DNA from Plant 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. The CTAB method can be used both for freeze-dried leaves and for fresh leaves. The scale of extraction is dependent on the amount of starting material, for e.g. 300–400 mg of material requires 9 ml of Extraction Buffer, which yields upto 500 µg of DNA.

Principle

CTAB (Cetyl trimethylammonium bromide), a detergent is used to break open and solubilize the contents of plant cells. Chlorophyll and some denatured proteins are removed from green plant tissue in an organic chloroform/octanol 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 redissolving in aqueous solution. The concentration of the DNA can be estimated by agarose gel electrophoresis. The DNA recovered is not free from contaminants such as carbohydrates, but is of suitable grade for enzyme digestion, southern blotting and analysis by PCR.

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 260nm, 280nm, and 320nm using a quartz microcuvette. Absorbance readings at 260nm should fall between 0.1 and 1.0. The 320nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260nm corresponds to approximately 50µg/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 260nm to absorbance at 280nm.

Concentration of DNA sample (µg/ml) = 50 x A_{260} x dilution factor.

Storage

Store the HiPurA® Plant DNA Isolation Kit (CTAB Method) between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 18 months.

Materials needed but not provided

- 2- mercaptoethanol (β -ME) (Product Code: MB041)
- Chloroform (Product Code: MB109)
- Octanol
- Isopropanol (Product Code: MB063)
- Ethanol (96 –100%)
- Ethanol (70%)
- 15ml Centrifuge Tube with screw cap (Product code: PW144)
- Chloroform: Isoamyl alcohol (24:1) (Product code: MB115)

Preparation Instructions

1. Grinding of the plant material can be done using mortar and pestle with CTAB Extraction Buffer. For plants such as maize and lettuce that have pronounced leaf midribs, these should be removed from the material before grinding, as it is a major source of carbohydrate contamination.
2. **Preparation of CTAB Extraction Buffer:** Immediately prior to use add, 90 μ l of β -mercapthoethanol and 90 mg CTAB powder in 9 ml of CTAB Extraction Buffer. Preheat the solution to 65°C.
3. Chloroform: Octanol (24:1). Store in dark at room temperature (15-25°C). Prepare and dispense this solution in a fume hood.
4. To the CTAB Wash Buffer Concentrate (120 ml) provided, add 360 ml of Ethanol (100%) prior to use.
5. Prechill the CTAB Wash Buffer and 70% ethanol 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 (15-25°C) and are given in g; the correct rpm can be calculated using the formula:

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

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/ 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

Protocol

1. To 300-400 mg of the ground tissue in a sterile centrifuge tube, add 9 ml of prewarmed CTAB Extraction Buffer (**Refer General Preparation Instructions**). Mix gently by inversion.

NOTE: Ensure that CTAB powder and β -mercaptoethanol is added to CTAB Extraction Buffer prior to use. DNA can be extracted from fresh plant tissue by grinding a leaf or leaf disc in a small amount of Extraction Buffer.

2. Incubate the samples for 60 - 90 minutes, with occasional inversion at 65°C.
3. Allow the samples to cool by keeping it at room temperature (15-25°C) for 5 minutes.
4. Add 5 ml of Chloroform: Octanol (24:1), and mix gently by inverting the tubes for 5 minutes.

NOTE: Chloroform: Isoamyl alcohol (24:1) can also be used instead of Chloroform: Octanol (24:1)

5. Spin the samples at 850 x g (\approx 3000 rpm) for 2 minutes at room temperature (15-25°C).
6. Transfer the top aqueous layer into a fresh tube and add 25 μ l of RNase A (20 mg/ml) (DS0003). Mix the sample gently by inversion and incubate for 30 minutes at room temperature (15-25°C).
7. Add 6 ml of isopropanol to each tube. Mix the samples gently by inversion until a white fluffy DNA precipitate appears (it should appear within 1 minute of addition of isopropanol).
8. Centrifuge at 850 x g (\approx 3000 rpm) for 5 minutes at room temperature (15-25°C) to pellet the DNA. Discard the supernatant.
9. Resuspend the pellet in 8 ml of cold diluted CTAB Wash Buffer (DS0004) (**Refer to Preparation Instructions**). Incubate at room temperature (15-25°C) for 20 minutes. Centrifuge at 850 x g (\approx 3000 rpm) for 5 minutes at room temperature (15-25°C). Discard the supernatant.
10. Add 8 ml of cold 70% ethanol to the tube containing DNA. Centrifuge at 850 x g (\approx 3000 rpm) for 5 minutes at room temperature (15-25°C). Discard the supernatant.
11. Air dry the pellet to remove the traces of ethanol for about 10 minutes.
12. Transfer the DNA sample to a tube containing 1 ml of Elution Buffer (ET). Dissolve the pellet gently by pipetting.

Storage of the eluate with purified DNA: For short-term storage (24-48 hrs) 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 will help to stabilize the DNA at these temperatures.

References

1. Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98, 503-517

- Tai, T.H. and Traksley, S.D. (1990) A rapid and in expensive method for isolation of total DNA from dehydrated plant tissue. *Plant Mol. Biol. Reprtr.* 8, 297-303
- Sanghai-Marooof, M.A., Soliman, K.M., Jorgensen, R.A., and Allard, R.W. (1984) Ribosomal DNA space-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl.Acad. Sci.USA* 81, 8014-8018.
- Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18, 6531-6535.

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

- 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 DNA Isolation Kit (CTAB Method) is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Yield	DNA Purity
Plant leaf	Upto 500 µg	1.6-1.9

Troubleshooting Guide

Sr. No.	Problem	Probable Cause	Solution
1.	Turbidity in DNA after overnight incubation at 4°C.	DNA not dissolved completely	Heat the sample to 65°C for 10 minutes and invert the tube every 3 minutes. Insoluble material remaining after this treatment can be removed by centrifugation at 850 x g (≈ 3000 rpm) for 5 minutes. The clear supernatant can be transferred into a fresh tube. The pellet can be discarded as it does not contain DNA.
2.	Debris in the tube containing the supernatant obtained after centrifuging at 850 x g (≈ 3000 rpm) for 2 minutes.	Improper pipetting	Spin the supernatant at 850 x g (≈ 3000 rpm) for 5 minutes.
3.	Purity of the DNA is lower than expected.	DNA is contaminated with RNA	25 µl of RNase A (20 mg/ml) can be added to the top aqueous layer obtained after centrifugation at 850 x g (≈ 3000

			rpm) for 2 minutes before the addition of 6ml isopropanol.
4.	Carbohydrate contamination in the sample.	Grinding of the midrib along with the leaf material	Remove the midrib from the leaf before grinding, mainly in case of maize and lettuce, as these plant species have pronounced midribs. Removal of the midrib is not important in case of very young leaves or in small grain cereals.
5.	DNA appears degraded (as a smear running down the gel).	During freeze drying, the plant material is not immediately frozen	While harvesting plant material for freeze drying, ensure that the tissue is immediately frozen, as this reduces DNA degradation.
		DNA might get 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 ET (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 resuspension and solubilization in ET (pH 8.5)

Safety Information

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.

Please refer disclaimer Overleaf.

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

Identification No.: PIMB502

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Disclaimer :

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