

MB561

HiPurA® Marine Algae DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB561		
		20 Preps	50 Preps	250 Preps
DS0093	STE Buffer	70 ml	175 ml	2 X 437.5 ml
DS0002	CTAB Extraction Buffer	6 ml	15 ml	75 ml
DS0003	RNase A Solution (20 mg/ml)	0.5 ml	1.25 ml	6.25 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	6 ml	15 ml	75 ml
DSCA02	HiShredder (in DBCA016 Collection tube)	40 nos	100 nos	2 X 250 nos

Intended Use

Recommended for isolation of DNA from Algal samples.

Introduction

DNA extraction from algae remains difficult due to the presence of a rigid cell wall that contains sulphated polysaccharides. Viscous soluble polysaccharides are released by grinding Algae material in liquid nitrogen that are difficult to separate from DNA, therefore CTAB method has been applied for DNA extraction. The scale of extraction is dependent on the amount of starting material.

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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 the plant tissue in an organic chloroform/Isoamyl 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 suitable for enzyme digestion, Southern blotting and analysis by PCR.

HiShredder (DSCA02)

For the plant genomic DNA isolation procedure, contaminants such as cell debris, salt precipitates are removed by centrifugation through a HiShredder. The HiShredder removes all cell debris and precipitates making the preparation of a clear lysate rapid and efficient. Preparation of the clear lysate is essential to prevent clogging of the HiElute Miniprep Spin Column (capped) used in the subsequent steps.

Elution

The yield of genomic DNA depends on the sample type and the number of cells in the sample. Elution with 200µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 200µl 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® Marine Algae 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

- Mortar and pestle
- Liquid nitrogen
- Chloroform:Isoamyl alcohol (24:1) (Product Code: MB115)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96-100%)
- β - mercapthoethanol (Product Code: MB041)
- Bovine Serum Albumin (Product Code: MB083)
- 70% Ethanol
- 3M Sodium acetate pH 5.2 (Product Code: ML009)
- 65°C water bath or heating block
- Molecular Biology Grade Water (Product Code: ML024)

Storage

Store the HiPurA® Marine Algae DNA Purification 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. Preheat a water bath or heating block to 65°C.
2. **Preparation of STE Buffer**
Add 2 µl of β - mercapthoethanol and 2 mg Bovine Serum Albumin (BSA) per ml of STE Buffer prior to use.
NOTE: STE buffer should be freshly prepared before the experiment.
3. **Thoroughly mix reagents**
Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.
4. Ensure that clean & dry tubes and tips are used for the procedure.
5. Pre-chill the mortar and pestle at -20°C.

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.

Sample Preparation (Manual Disruption with mortar and pestle)

Weigh upto 250 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.

NOTE: Delay in DNA isolation after sample preparation will result in DNA degradation and reduced yield.

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 the ground material, immediately add 3 ml of STE Buffer (with β - mercapthoethanol and Bovine Serum Albumin) and mix thoroughly. [Do not grind the plant material after the addition of STE Buffer, as it may cause shearing of DNA]. **Proceed immediately to step 2 without delay.**

NOTE: For the preparation of STE Buffer, refer General Preparation Instructions.

2. **Load sample in HiShredder (DSCA02)**

Add the sample to the HiShredder placed in a 2.0 ml collection tube and centrifuge for 2 minutes at a maximum speed (≈13,000 rpm). Discard the flow-through fraction without disturbing the cell pellet.

NOTE: The above step should be performed in two different HiShredders (provided) as the entire sample cannot be processed in the same HiShredder.

3. To each pellet, add 200 μl of CTAB Extraction Buffer, mix thoroughly and pool the contents of both the tubes into a single tube (not provided).

Optional RNase A treatment

If RNA-free genomic DNA is required, add 20 µl of RNase A Solution (DS0003), mix and incubate for 10 minutes at room temperature (15-25°C), then continue with step 5.

4. Incubate the samples at 65°C for 30 minutes.
5. Add 400 µl of Chloroform: Isoamyl alcohol (24:1) (Product Code: MB115). Mix the sample gently by inversion for 10 minutes. Centrifuge for 10 minutes at a maximum speed (≈13,000 rpm).
6. Following centrifugation, mixture separates into three phases: lower organic phase, an interphase containing debris and an upper aqueous phase containing DNA.
7. Transfer the top aqueous layer into a fresh tube. Add 2 volumes of ethanol (96-100%) and 0.1 volumes of 3M Sodium acetate, pH 5.2; mix gently.
8. Incubate the samples at -20°C for 10 minutes. Centrifuge for 10 minutes at a maximum speed (≈13,000 rpm). Discard the supernatant.
9. Resuspend the pellet in 500 µl of 70% ethanol and centrifuge for 10 minutes at a maximum speed (≈13,000 rpm). Discard the supernatant.
10. Air dry the pellet to remove traces of ethanol.

NOTE: Do not over-dry the pellet.

11. Dissolve the pellet gently in 200 µl of Elution Buffer (ET) (DS0040) by pipetting.

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.

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

Quality Control

Type of Sample	DNA Purity
Red Algae	1.6-1.9

Troubleshooting guide

Sr. No.	Problem	Possible 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 13,000 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 ≈13,000 rpm for 2 minutes	Improper pipetting	Spin the supernatant at 13,000 rpm for 5 minutes.
3.	Purity of the DNA is lower than expected	DNA is contaminated with RNA	After the addition of 200 µl CTAB Extraction buffer, 20 µl of RNase A (20 mg/ml) can be added to the pellet (obtained after centrifugation at 13,000 rpm for 2 minutes).
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).

Please refer disclaimer Overleaf.

Safety Information

HiPurA® Marine Algae 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



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



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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 Website: www.himedialabs.com