

MB574

HiPurA[®] 96 SuperPlant DNA Purification Kit

Kit Contents

| Product Code | Reagents provided | MB574 |
|--------------|---|------------|
| | | 1X96 Preps |
| DS0200 | SuperPlant Extraction Buffer | 100 ml |
| DS0070 | Additive-II | 10 ml |
| DS0071 | Additive-III | 2 g |
| DS0019 | Wash Solution Concentrate (WSP) | 45 ml |
| DS0040 | Elution Buffer (ET) [10mM Tris-Cl, pH 8.5] | 12 ml |
| DS0003 | RNase A Solution (20 mg/ml) | 2.5 ml |
| DBPL-96-01 | HiPurA [®] 96-well DNA Plate | 1 no. |
| LWB-96 | HiPurA [®] 96-well Block (2.2 ml) | 1 no. |
| LWB-16-96 | HiPurA [®] 96-well Block (1.6 ml) | 2 nos. |
| PR11 | HiPurA [®] Silicon Pad for sealing | 1 no. |
| DVB-96 | HiPurA [®] 96-well V-Block | 1 no. |

Introduction

The HiPurA[®] 96 SuperPlant DNA Purification Kit provides a fast and easy method for purification of total DNA from plant for reliable applications in PCR and Southern blotting technique etc. The DNA purification procedure using the 96-well format comprises of three steps viz. adsorption of DNA to the membrane, removal of residual contaminants and elution of pure genomic DNA. The scale of extraction is dependent on the amount of starting material, for e.g. 200 mg of material requires 900 µl of SuperPlant Extraction Buffer and yields 10-50 µg of DNA. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

HiPurA[®] 96 SuperPlant DNA Purification Kit

This kit simplifies isolation of DNA from fresh plant material with spin column procedure. The procedure is optimized for a maximum of 200 mg of wet-weight of the starting material. The sample (fresh) is cut and ground in liquid nitrogen along with SuperPlant Extraction Buffer. SuperPlant 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 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.

HiPurA® 96-well DNA Plate (DBPL-96-01)

HiPurA® 96-well DNA Plate is based on the advanced silica binding principle presented in a centrifugation and vacuum format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced silica membrane to yield high quantity of DNA. It facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. DNA binds specifically to the advanced silica-gel membrane while contaminants pass through. PCR inhibitors such as divalent cations and proteins are completely removed during wash steps, leaving pure nucleic acids to be eluted in the buffer provided with the kit.

Elution

The yield of plant 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 reactions. 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 upto 20-30 kb, and is suitable for direct use in PCR, restriction digestion, and 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 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® 96 SuperPlant DNA Purification Kit is free of protein and other contaminants that can inhibit enzymatic reactions or any downstream applications.

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

Materials needed but not provided

- Mortar and pestle
- Tabletop Centrifuge with 96-well rotor and plate carriers, capable of attaining atleast 5,000 rpm or Vacuum Manifold for 96 well plate
- Vacuum source regulator (pump) (capable of giving negative pressure of 25 to 30 inches Hg)
- Vacuum regulator
- Multi-channel pipette with tips
- Ethanol (96-100%)
- Water bath or heating block at 65°C
- Hot air oven at 70°C
- Liquid Nitrogen
- Chloroform: Isoamylalcohol (24:1)(Product Code: MB115)

Storage

Store the HiPurA® 96 SuperPlant 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. Grinding of the plant material can be done using mortar and pestle. Midrib should be removed from the material before grinding, as midrib is a major source of carbohydrate contamination.
2. Prechill the mortar and pestle to –20°C before use.

- SuperPlant Extraction Buffer:** Immediately prior to use, add 90 µl of Additive-II and 18 mg of Additive-III in 900 µl of SuperPlant Extraction Buffer. Preheat the solution to 65°C.
- Dilute Wash Solution Concentrate (WSP) (DS0019) as follows:**

| Number of Preps | Wash Solution Concentrate (WSP) | Ethanol (96-100 %) |
|-----------------|---------------------------------|--------------------|
| 1 X 96 PR | 45 ml | 105 ml |

- Preheat a water bath or heating block to 65°C and 70°C.
- 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.

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 conventional laboratory centrifuge e.g. Beckman CS-6KR, Heraeus Varifuge 3.0R, or Sigma 6k10 with swinging bucket rotors with adaptors and carriers for 96-well format. The 96-well accessories provided with the kit are compatible with almost all laboratory centrifuges and rotors. 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.

General Note:

- Vacuum protocol as well as centrifugation protocol can be performed for 96 SuperPlant DNA isolation. For a vacuum protocol, negative pressure of 30 mm Hg is required to achieve optimum results.
- The HiPurA® Silicon Pad for sealing (PR11) used in the protocol should not be discarded. It can be reused after wiping with ethanol and washing properly.

DNA Isolation Protocol

For a 96-well format, processing of multiple samples at the same time is not feasible. Delay in continuing of DNA isolation may result in degradation of DNA and thus lower yield. Disruption and homogenization of plant tissue using mortar and pestle in liquid nitrogen is recommended.

Sample preparation (Grinding):

For Leaf Sample: Finely cut the leaf material before grinding. Midrib should be removed before cutting the leaves as midrib is a major source of carbohydrate contamination. Weigh 200 mg (each well) 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: Midrib should be removed from the leaf material before grinding, as the midrib is a major source of carbohydrate contamination.

This kit is optimized for leaf tissues; however, it can also be used with other plant tissues.

Procedure

Vacuum based protocol:

NOTE: Ensure that Additive-II and Additive-III are added to SuperPlant Extraction Buffer (DS0200) as mentioned in General Preparation Instructions.

1. To each well of HiPurA® 96-well Block (2.2 ml) (LWB-96) containing ground plant material (200 mg), immediately add 900 µl of SuperPlant Extraction Buffer (DS0200) (preheated to 65°C) (Refer General Preparation Instructions)
2. Seal the HiPurA® 96-well Block (2.2 ml) (LWB-96) using the provided HiPurA® Silicon Pad for sealing (PR11) and mix vigorously by vortexing.
3. Incubate the samples for 60-90 minutes with occasional inversion at 65°C.
4. Add 700 µl of Chloroform: Isoamylalcohol (24:1) and mix gently by pipetting. Seal the HiPurA® 96-well Block (2.2 ml) (LWB-96) using the provided HiPurA® Silicon Pad for sealing (PR11)
5. Centrifuge the 96-well DNA Block (2.2 ml) along with the sample at 3,700 rpm for 10 minutes.
6. Transfer the top aqueous layer (containing DNA) into a HiPurA® 96-well Block (1.6 ml) (LWB-16-96) and add 20 µl of RNase A Solution (20 mg/ml) (DS0003). Incubate for 5 minutes at room temperature (15-25°C).
7. Add equal volume of Ethanol (96-100%) to the lysate obtained from the above step and mix by pipetting.
8. Add 650µl of lysate from step 7, including any precipitate, which may have formed, to the HiPurA® 96-well DNA Plate (DBPL-96-01) placed in HiPurA® 96-well Block (1.6 ml) (LWB-16-96).
9. Connect the vacuum manifold (LA664) to the vacuum source. Remove the manifold top and place a HiPurA® 96-well Block (1.6 ml) (LWB-16-96) into the manifold base to collect the flow-through liquid. Replace the manifold top and place the HiPurA® 96-well DNA Plate (DBPL-96-01) onto the manifold top. Seal it with HiPurA® Silicon Pad for sealing (PR11).

NOTE: Make sure that the vacuum manifold can give a negative pressure of 30 inches Hg .We recommend the use of a vacuum regulator to adjust the negative pressure.

NOTE: -30 inches Hg is equivalent to approximately 1000 mbar and 15 psi

10. Turn on the vacuum source and adjust it to achieve -30 inches Hg of pressure. Continue to draw vacuum through the plates until no liquid remains in any of the wells of HiPurA® 96-well DNA Plate (DBPL-96-01). Turn off the vacuum source and release the vacuum from inside of the vacuum manifold using the vacuum regulator. Remove the HiPurA® 96-well DNA Plate (DBPL-96-

01) from the manifold and temporarily set it aside on a piece of absorbent toweling (tissue paper stacks) or plastic wrap.

NOTE: Discard the flow-through liquid from HiPurA® 96-well Block (1.6 ml) (LWB-16-96) and reuse the block.

11. Repeat steps 8-10 with the remaining sample. Discard the flow-through liquid.

12. Wash

(Prepare the Wash Solution Concentrate (WSP) (DS0019) as indicated in General Preparation Instructions)

Add 500 µl of diluted Wash Solution (WSP) (DS0019) to each well of HiPurA® 96-well DNA Plate (DBPL-96-01). To seal the plate, cover it with HiPurA® Silicon Pad for sealing (PR11). Continue to draw vacuum until no Wash Solution is present in any of the wells. Discard the flow-through liquid and reuse the block.

13. Add another 500 µl of the diluted Wash Solution (WSP) to each well of HiPurA® 96-well DNA Plate (DBPL-96-01). Draw vacuum until no Wash Solution is present in any of the wells.

14. Discard the flow-through liquid, reuse the block and apply vacuum for 10 minutes at negative pressure 30 inches of mercury to remove the traces of ethanol present in the Wash Solution.

15. Turn off the vacuum source and release the vacuum. Remove the HiPurA® 96-well DNA Plate (DBPL-96-01) from the vacuum manifold and tap the plate approximately 6-8 times on several layers of absorbent toweling (tissue paper stacks). Be careful not to damage the drip directors on the underside of the plate.

NOTE: Lint-free absorbent toweling is recommended to avoid the release of tiny fibres, which could contaminate the genomic DNA and interfere with subsequent capillary electrophoresis.

16. Vacuum Oven Drying

Incubate HiPurA® 96-well DNA Plate (DBPL-96-01) for 15 minutes at 70°C in a hot air oven along with vacuum to evaporate residual ethanol.

17. DNA Elution

Remove the HiPurA® 96-well Block (1.6 ml) (LWB-16-96) which was used as collection tray from the vacuum manifold base and replace it with a new HiPurA® 96-well V-Block (DVB-96) for elution. Reassemble the manifold top and place the HiPurA® 96-well DNA Plate (DBPL-96-01) on to it. Add 100 µl of Elution Buffer (ET) to the corresponding wells of HiPurA® 96-well DNA Plate (DBPL-96-01) and incubate at room temperature (15-25°C) for 5 minutes. Turn on the vacuum source and allow the vacuum to continue for 5 minutes.

NOTE: Make sure that the vacuum manifold gives a negative pressure of 30 inches Hg.

18. Turn off the vacuum source and use the vacuum regulator to gradually release the vacuum from the manifold. Disassemble the manifold and remove the HiPurA® 96-well V-Block (DVB-96) which contains eluted genomic DNA samples.

Centrifugation based Protocol:

This procedure requires the use of a high-speed centrifuge with a 96-well rotor and plate carriers.

NOTE: Ensure that Additive-II and Additive-III are added to SuperPlant Extraction Buffer (DS0200) as mentioned in General Preparation Instructions.

1. To each well of HiPurA® 96-well Block (2.2 ml) (LWB-96) containing ground plant material (200 mg), immediately add 900 µl of SuperPlant Extraction Buffer (DS0200) (preheated to 65°C) (Refer General Preparation Instructions)
2. Seal the HiPurA® 96-well Block (2.2 ml) (LWB-96) using the provided HiPurA® Silicon Pad for sealing (PR11) and mix vigorously by vortexing.
3. Incubate the samples for 60-90 minutes with occasional inversion at 65°C.
4. Add 700 µl of Chloroform: Isoamylalcohol (24:1) and mix gently by pipetting. Seal the HiPurA® 96-well Block (2.2 ml) (LWB-96) using the provided HiPurA® Silicon Pad for sealing (PR11)
5. Centrifuge the 96-well DNA Block (2.2 ml) along with the sample at 3,700 rpm for 10 minutes.
6. Transfer the top aqueous layer (containing DNA) into a HiPurA® 96-well Block (1.6 ml) (LWB-16-96) and add 20 µl of RNase A Solution (20 mg/ml) (DS0003). Incubate for 5 minutes at room temperature (15-25°C).
7. Add equal volume of Ethanol (96-100%) to the lysate obtained from the above step and mix by pipetting.
8. Add 650 µl of lysate from step 7, including any precipitate, which may have formed, to the HiPurA® 96-well DNA Plate (DBPL-96-01) placed in HiPurA® 96-well Block (1.6 ml) (LWB-16-96).
9. Centrifuge for 5 minutes at 3,700 rpm at room temperature (15-25°C). Discard the flow-through and reuse the block.
10. Repeat step 8-9 with the remaining sample. Discard the flow-through liquid and reuse the block.
11. **Wash**
(Prepare the Wash Solution as indicated in General Preparation Instructions)
 Add 500 µl of diluted Wash Solution (WSP) (DS0019) to each well of HiPurA® 96-well DNA Plate (DBPL-96-01) placed on the 1.6 ml block and centrifuge for 10 minutes at 3,700 rpm at room temperature (15-25°C).

NOTE: Discard the flow-through and reuse the block.
12. Add another 500 µl of the diluted Wash Solution (WSP) to each well of the HiPurA® 96-well DNA Plate (placed on the 1.6 ml block). Centrifuge for 10 minutes at 3,700 rpm at room temperature (15-25°C) and discard the flow-through and reuse the block.
13. Centrifuge the HiPurA® 96-well DNA Plate (placed on the 1.6 ml block) for an additional 10 minutes at 3,700 rpm at room temperature (15-25°C) to dry the membrane.
14. The HiPurA® 96-well DNA Plate can be incubated at 70°C in hot air oven for 15 minutes to remove the traces of ethanol present in the Wash Solution.
15. **DNA Elution**
 Place the HiPurA® 96-well DNA Plate on a new HiPurA® 96-well V-Block (DVB-96). Pipette 100 µl of the Elution Buffer (ET) (DS0040) directly into each well of HiPurA® 96-well DNA Plate. Incubate for 5 minutes at room temperature (15-25°C). Centrifuge at 3,700 rpm for 5 minutes at room temperature 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® 96 SuperPlant DNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

| Type of Sample | DNA Yield | DNA Purity |
|----------------------------|----------------|------------|
| Plant leaf sample (100 mg) | 1-15 µg of DNA | 1.6-1.9 |

Troubleshooting guide

| Sr. No. | Problem | Possible Cause | Solution |
|---------|---|--|--|
| 1. | Clogged HiPurA® 96-well DNA Plate | Carryover of the particulate material | Ensure that no particulate material is transferred through the HiPurA® 96-well DNA Plate. |
| | | Lysate is too viscous | The amount of starting material can be reduced or the amount of SuperPlant Extraction Buffer can be increased. |
| | | Insufficient centrifugation | The g-force and the centrifugation time can be increased. |
| 2. | Lower yields of DNA | Insufficient disruption of the plant tissue | Ensure that the plant material is disrupted in sufficient amounts of liquid nitrogen (if grinding using liquid nitrogen). It is very important that the disrupted tissue sample should not thaw before addition of SuperPlant Extraction Buffer. |
| | | Insufficient lysis | The amount of starting material can be reduced or the amount of SuperPlant Extraction Buffer can be increased. |
| | | Improper binding | Ensure that the binding conditions are adjusted correctly by accurately determining the amount of lysate recovered. |
| 3. | Darkly colored membrane or green/yellow eluate after washing with wash solution | Insufficient washing of the membrane | After washing with the Wash Solution (WSP), an additional wash with 500 µl ethanol (96-100%) should be performed. Drying of the plate is important before elution. |
| | | Amount of starting material is more than recommended | Reduce the amount of starting material for future preps |
| 5. | Poor performance of DNA in downstream | Ethanol carryover | Ensure that after the second wash with Wash Solution (WSP), drying of the plate is done (as mentioned in the procedure). Also the DNA plate should not come in contact with the flow-through liquid. |

| | | | |
|--|-------------|----------------|--|
| | experiments | Salt carryover | Ensure that the Wash Solution is at room temperature (15–25°C) before use. |
|--|-------------|----------------|--|

Safety Information

HiPurA® 96 SuperPlant 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.

Please refer disclaimer Overleaf.



Storage temperature



Do not use if package is damaged



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02/2025

PIMB574_0/0222

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Disclaimer :

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