# MB542 HiPurA® Soil DNA Purification Kit

## Kit Contents

| Product Code | Reagents provided | MB542 | | |
|---|---|---|---|---|
| | | 20 Preps | 50 Preps | 250 Preps |
| DS0066 | Inhibitor Removal Solution (IRSH) | 8 ml | 20 ml | 100 ml |
| DS0079 | Soil Lysis Solution (SL) | 18 ml | 45 ml | 225 ml |
| DS0067 | Binding Solution (SB) | 28 ml | 70 ml | 350 ml |
| DS0019 | Wash Solution Concentrate (WSP) | 8 ml | 20 ml | 100 ml |
| DS0040 | Elution Buffer (ET) [10mM Tris-Cl, pH 8.5] | 4 ml | 10 ml | 50 ml |
| DBCA04 | HiBead Tubes | 20 no. | 50 no. | 250 no. |
| DBCA03 | HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube] | 20 no. | 50 no. | 250 no. |
| DBCA016 | Collection Tube (Uncapped), Polypropylene (2.0 ml) | 20 no. | 50 no. | 250 no. |
| PW1139 | Collection Tube, Polypropylene (2.0 ml) | 40 no. | 100 no. | 2 x 250 no. |

## Intended Use

Recommended for isolation of DNA from Soil samples.

## Introduction

HiPurA® Soil DNA Purification Kit provides a fast and easy method for purification of total DNA from environmental samples containing a high humic acid content including difficult soil types such as compost, sediment, manure and other common soil types, for reliable applications in PCR and other downstream applications. The HiPurA® DNA purification system combines the reversible nucleic acid binding properties of HiElute Miniprep Spin Column (Capped) with an efficient buffer system, which eliminates PCR inhibiting compounds such as humic acid from soil samples.

## HiPurA® Soil DNA Purification Kit

HiPurA® Soil DNA Purification Kit simplifies isolation of DNA from soil using spin column technique. The kit is optimized to process 250-500 mg of soil sample. Soil samples possess typically many compounds that can inhibit downstream enzymatic reactions. The HiPurA® Soil DNA Purification Kit effectively removes these inhibitory compounds during the extraction process. The Inhibitor Removal Solution (IRSH), provided in the kit, is effective in removing PCR inhibitors from even the most difficult soil types.

The soil sample is lysed and homogenized in a bead-beating step. The total genomic DNA obtained is bound to silica membrane in a spin column format, washed and eluted from the membrane. The purified DNA can directly be used for PCR analysis and other downstream applications.

## 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 silica gel membrane and the speed plus versatility of spin column technology to yield high quantity of DNA.



ISO 9001-2015 CERTIFIED WHO GMP CERTIFIED ISO 13485-2016 CERTIFIED

Registered Office
HiMedia Laboratories Pvt Ltd.
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

Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

The information contained herein is believed to be accurate and complete. However no warranty or guarantee whatsoever is made or is to be implied with respect to such information or with respect to any product, method or apparatus referred to herein

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 isolation 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.

**Elution**

The yield of genomic DNA depends on the sample type and the number of microorganisms present in the sample. A single elution with 100 µl of Elution Buffer (ET) (DS0040) or Molecular Biology Grade Water (ML024) will provide sufficient DNA to carry out multiple amplification reaction. The eluted DNA is suitable for direct use in PCR, restriction digestion, and hybridization techniques.

**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® Soil 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**

- Ethanol (96 - 100 %)
- Flat-bed vortex
- Tabletop Microcentrifuge [capable of atleast 13,000 x g (≈14,000 rpm)]
- Molecular Biology Grade Water (Product Code: ML024)
- Water bath preheated to 95°C (Optional)
- Water bath or heating block preheated to 60°C (Optional)

**Storage**

Stored at HiPurA® Soil 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 the water bath or heating block to 60°C or 95°C as required.
2. **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.
3. Ensure that clean & dry tubes and tips are used for the procedure.

4. **Dilute Wash Solution Concentrate (WSP) (DS0019) as follows:**

| Number of Preps | Wash Solution Concentrate (WSP) | Ethanol (96-100%) |
|---|---|---|
| 20 | 8 ml | 19 ml |
| 50 | 20 ml | 47 ml |
| 250 | 100 ml | 235 ml |

**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. The tubes 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.

**Specimen Collection and Handling**

Collect soil sample in a sterile container and store the sample at 2-8°C for short term storage or -20°C for long term storage.

**Types of Specimen**

Samples: Soil, ash

**Procedure**

**NOTE:** 1) The regular protocol given below is based on bead beating for maximum yield of DNA. However, for sensitive applications where the shearing of DNA is of concern, instead of vortexing the HiBead tube for 10 minutes, the tube can be incubated at 95°C in water bath for 10 minutes, with intermittent mixing of the contents of the tube every 2-3 minutes by pulse vortexing or inversion.

2) The soil sample can also be ground in liquid nitrogen using motor and pestle before adding Soil Lysis Solution (SL) for soil samples which are difficult to lyse.

**NOTE: <u>FOR DNA PURIFICATION FROM DIFFICULT SOIL SAMPLES, FOLLOW ALTERNATIVE PROTOCOL</u>**

1. **Lysis**
   To the HiBead Tube (DBCA04) provided, add 750 µl of Soil Lysis Solution (SL) (DS0079) and 250-500 mg of soil sample. Mix by inverting the tube several times or by gentle vortexing.

2. Secure the HiBead tube horizontally on a flat-bed vortex pad using a tape and vortex at maximum speed for 10 minutes.

   **NOTE:** Ensure that the HiBead tubes rotate freely in the centrifuge without rubbing.

3. Centrifuge the tube at 13,000 x g (≈14,000 rpm) for 1 minute at room temperature.

   **NOTE:** Make sure not to exceed the speed more than 13,000 x g or else the tubes may break.

4. Transfer the supernatant to a new capped 2.0 ml collection tube.

   **NOTE:** The supernatant may still contain some soil particles.

5. Add 250 µl of Inhibitor Removal Solution (IRSH) (DS0066), vortex for 5 seconds and incubate at 4°C for 5 minutes.

6. Centrifuge the tube for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature.

7. **Binding**
   Transfer the supernatant to a clean 2.0 ml collection tube (Not Provided); add 1.2 ml of Binding Solution (SB) (DS0067) and vortex for 5 seconds.

8. **Load onto HiElute Miniprep Spin Column (Capped) (DBCA03)**
   Load approximately 650 µl of the lysate on the HiElute Miniprep Spin Column (Capped) and centrifuge for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature. Discard the flow-through. Repeat the above step with the remaining sample. Discard the flow-through liquid and reuse the 2.0 ml collection tube (uncapped).

9. **Wash**
   **(Prepare Wash Solution as indicated in General Preparation Instructions)**
   Add 500 µl of diluted Wash Solution (WSP) (DS0019) and centrifuge for 1 minute at 6000 x g (≈8000 rpm).

   **NOTE:** Discard the flow-through and reuse the 2.0 ml collection tube (uncapped).

10. Add another 500 µl of diluted Wash Solution (WSP) to the column and centrifuge for 2 minutes at a maximum speed (≈14,000 rpm). Discard the flow-through and reuse the same collection tube.

11. **DNA Elution**
    Transfer the column to a new 2.0 ml collection tube (uncapped) and add 100 µl of Elution Buffer (ET) (DS0040) or Molecular Biology Grade Water (ML024) directly onto the center of the column membrane. Centrifuge the tube for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature. Transfer the eluate to a new capped 2.0ml collection tube for DNA storage.

**Alternative Protocol (for difficult soil samples)**

1. To the HiBead Tube (DBCA04) provided, add 750 µl of Soil Lysis Solution (SL) (DS0079) and 250-500 mg of soil sample. Mix by inverting the tube several times or by gentle vortexing.

2. Secure the HiBead tube horizontally on a flat-bed vortex pad using a tape and vortex at maximum speed for 20 minutes.

3. Incubate the HiBead tube at 60°C for 20 minutes.

4. After incubation, secure the HiBead tube horizontally on a flat-bed vortex pad using a tape and vortex at maximum speed for another 20 minutes.

5. Continue from step 3 of the Regular Protocol.

**Storage of the eluate with purified DNA:** The eluate contains pure soil 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 denaturation of DNA. The Elution Buffer 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® Soil DNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

**Quality Control**

| Type of Sample | DNA Yield | DNA Purity |
|---|---|---|
| Soil sample | Upto 20 µg | 1.6-1.9 |

**Troubleshooting guide**

| Sr. No. | Problem | Possible Cause | Solution |
|---|---|---|---|
| 1 | Low genomic DNA yield or no DNA eluted | Poor homogenization of sample | Depending on the type of soil, further vortexing with the flat bed vortex or bead beater equipment may be required. However, it is not recommended to increase the vortex time to longer than 10 minutes at maximum speed. Also, ensure that the maximum input of 500 mg of soil is not exceeded, as this may also cause incomplete homogenization. |
| | | Ethanol was not added to the Wash Solution Concentrate (WSP) | Ensure that ethanol (96 - 100%) is added to the supplied Wash Solution Concentrate (WSP), prior to use (as mentioned in General Preparation Instructions). |
| 2 | Poor performance of DNA in downstream applications | DNA was not washed twice with the provided Wash Solution (WSP) | Traces of salt from the binding step may remain in the sample if the column is not washed twice with the provided Wash Solution (WSP). Salt may interfere with downstream applications, and thus must be washed from the column. |
| | | Ethanol carryover | Ensure that after the second wash with Wash Solution (WSP), the column is centrifuged for an additional minute at 12,000 x g (≈13,000 rpm), as mentioned in step 10. |
| | | PCR reaction conditions need to be optimized | 1) Take steps to optimize the PCR conditions being used, including varying the amount of template, changing the source of Taq polymerase, looking into the primer design and adjusting the annealing conditions.<br><br>2) A variety of PCR additives and enhancing agents can be used to increase the yield, specificity and consistency of PCR reactions. These include DMSO, formamide, glycerol, BSA etc. |

| | | | |
|---|---|---|---|
| | | | 3) Optimize cycling conditions: Decrease the annealing temperature of the cycling profile by 2 degrees or more. Some primer pairs require a lower annealing temperature (less stringent conditions) while amplifying soil DNA. |
| | | Too much DNA inhibits PCR reaction | Use the diluted DNA sample for downstream application if possible. |
| 3 | Clogged HiElute Miniprep Spin Column (Capped) | Insufficient centrifugation | The g-force and the centrifugation time can be increased. |

**Safety Information**

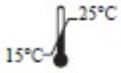
The HiPurA® Soil DNA Purification Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding 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

12/2024



Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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