**MB525** **HiPurA™ Bone DNA Extraction Kit**

## Kit Contents

| Product Code | Reagents provided | MB525 | |
|---|---|---|---|
| | | 10 Preps | 20 Preps |
| DS0075 | Bone Extraction Buffer | 105 ml | 205 ml |
| DS0076 | Binding Buffer (BBH) | 250 ml | 450 ml |
| DS0006 | Glass Powder Suspension (GPS) | 1.5 ml | 2.5 ml |
| DS0077 | Wash Solution Concentrate (BW) | 12 ml | 22 ml |
| DS0040 | Elution Buffer (ET) [10mM Tris-Cl, pH 8.5] | 5 ml | 10 ml |

## Introduction

HiPurA Bone DNA Extraction Kit provides a fast and easy method for isolation of genomic DNA from bone samples of various species (including forensic samples). The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

## HiPurA™ Bone DNA Extraction Kit

This kit simplifies the isolation of DNA from bone. Once the bone sample is decalcified, it is subjected to lysis by Bone Extraction Buffer. Following the lysis step is the binding of DNA. Two rapid wash steps remove trace salt and protein contaminants resulting in the elution of high quality DNA in the Elution Buffer provided with the kit.

## Elution

The yield of bone DNA depends on the sample type. A single elution with 100 µl of Elution Buffer will provide sufficient DNA to carry out multiple amplification reactions. The eluted DNA is suitable for direct use in PCR, restriction digestion, and Southern blotting applications.

## Concentration, yield and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of Bone DNA. Use Elution Buffer 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 Bone DNA Extraction 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:

- Razor blades and/or sandpaper
- Liquid Nitrogen
- Rotator at 56°C
- 15 ml sterile polypropylene tube
- 50 ml sterile polypropylene tube
- 2.0 ml Collection Tube
- Motar and pestle or freezer mill
- Parafilm
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Molecular Biology Grade Water (Product Code: ML024)



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**Storage**

HiPurA Bone DNA Extraction Kit can be stored at room temperature (15-25ºC) for upto 1 year without showing any reduction in performance. The product as supplied is stable at room temperature (15-25ºC).

**General Preparation Instructions**

1. **Thoroughly mix reagents**
   Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65ºC until the precipitate dissolves and allow cooling to room temperature (15-25ºC) before use.

2. **Dilute Wash Solution Concentrate (BW) (DS0077) as follows:**

| Number of Preps | Wash Solution Concentrate (BW) | Ethanol (96-100 %) |
|---|---|---|
| 10 | 12 ml | 12 ml |
| 20 | 22 ml | 22 ml |

3. Ensure that clean & dry tubes and tips are used for the procedure.

4. **Glass Powder Suspension (GPS):** Store tightly sealed with parafilm at 2-8°C. Mix well before using. If the Glass Powder suspension loses liquid and dries out, add sterile Molecular Biology Grade Water (ML024) such that the glass powder suspension accounts for approximately two-thirds of the total volume.

**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.

**Procedure**

**A. <u>Procedure for Decalcification of Bone</u>**

**<u>DAY 1</u>**

1. Completely remove bone marrow and soft tissues using razor blades and/or sandpaper. Before protocol soak the bone sample at 95ºC water bath overnight prior to clean. This method will make the cleaning easier.

2. Crush the bone into small fragments. Grind it to a fine powder using liquid nitrogen. If necessary use a freezer mill.

   **NOTE:** It is important to grind the bone fragments into a fine powder, since, finer the powder more is the DNA is released. However, if you use a freezer mill do not overgrind, as this may fragment the DNA.

3. Weigh not more than 500 mg of bone sample and transfer it to a sterile 15 ml polypropylene tube.

   **NOTE:** The bone sample powder can be stored at room temperature (15-25ºC), but we recommend to perform the extraction as soon as possible.

4. Add 10 ml of Bone Extraction Buffer (DS0075) to 500 mg of bone sample powder to decalcify the sample. Seal the tubes with parafilm and agitate the tubes overnight in dark at room temperature (15-25ºC) with gentle agitation on a rotator.

**B. Bone DNA Isolation Procedure**

**DAY 2**

5. To improve DNA yields, following the overnight agitation, incubate the samples with agitation for an additional 1-3 hours at 56°C.

   **NOTE:** If the powder is very fine, this step can be omitted, as it can cause further damage or degradation of DNA owing to high incubation temperature.

6. Centrifuge the sample for 2 minutes at 4,500 X g [≈ 5,000 rpm]. Transfer the supernatant to a 50 ml polypropylene tube containing 20 ml Binding Buffer (BBH) (DS0076).

7. Add 100 µl Glass Powder Suspension (GPS) (DS0006) to the tube and adjust the pH of the lysate to 4.0.

   **NOTE:** Glass Powder Suspension needs to be vortexed before pipetting, as the particles settle down quickly.

8. Close the tubes and seal with parafilm. Incubate the tube at room temperature (15-25°C) with agitation for 1 hour in dark.

9. Centrifuge the sample at 4,500 X g [≈ 5000 rpm] for 10 minutes. Discard the supernatant.

10. Add 1 ml of Binding Buffer (BBH) (DS0076) to the silica pellet and resuspend the pellet by pipetting up and down. Transfer the buffer- silica suspension into a fresh 2.0 ml collection tube.

11. Centrifuge the sample at 16,000 X g [≈ 13,000 rpm] for 1 minute. Discard the supernatant completely.

    **NOTE:** If the Binding Buffer is not completely removed, the salt concentration in the Elution Buffer will be too high and all DNA will not be released from the silica during elution.

12. Add 1 ml of diluted Wash Solution (BW) (DS0077) to the silica pellet and resuspend the suspension by pipetting up and down.

    **NOTE:** Prepare diluted Wash Solution (BW) as indicated in General Preparation Instructions.

13. Centrifuge the sample at 16,000 X g [≈ 13,000 rpm] for 1 minute. Discard the supernatant.

14. Add another 1 ml of diluted Wash Solution (BW) (DS0077) and centrifuge at 16,000 X g [≈ 13,000 rpm] for 1 minute. Discard the supernatant.

15. Dry the silica pellet for 15 minutes at room temperature (15-25°C).

    **NOTE:** Do not cap the tubes during the drying step.

16. Add 100 µl Elution Buffer (ET) (DS0040) to the dried silica pellet and resuspend by pipetting up and down.

17. Incubate the sample for 10 minutes at room temperature (15-25°C) with gentle occasional inversion.

18. Centrifuge at 16,000 X g [≈ 13,000 rpm] for 2 minutes.

19. Transfer the supernatant containing DNA into a fresh tube.

    **NOTE:** To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer (ET), then centrifuge. Elution with volumes less than 100 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. Storing

DNA in water may cause acid hydrolysis. Try to avoid transferring large amounts of silica, as it may interfere in downstream applications.

Storage of the eluate with purified DNA: The eluate contains pure bone 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. Sambrook, J.,*et al*. Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989)
2. Birren, B. and Lai, E. Pulsed Field Gel Electrophoresis: A practical guide (Academic Press, San Diego, CA, 1993)


**Troubleshooting guide:**

| Sr.No | Problem | Possible Cause | Solution |
|---|---|---|---|
| 1. | Purity of the DNA is lower than expected; ($A_{260}/A_{280}$ ratio is low) | Eluate was diluted in water for absorbance measurement | Use Elution Buffer (ET) provided. |
| | | Background reading is high due to silica fines | Spin the DNA sample at maximum speed for 1 minute, the supernatant can be used to repeat the absorbance readings. |
| 2. | Shearing of bone DNA | Improper handling of bone DNA | All pipetting steps should be executed as gently as possible. Wide orifice pipette tips are recommended to eliminate shearing of the DNA to a large extent. If the isolated DNA is to be used for PCR, instead of vortexing mix with gentle pipetting or invert until homogenous. This reduces shearing of DNA considerably. |
| 3. | Downstream applications are inhibited | Traces of ethanol present in the final bone DNA preparation | After the washing step spin the tube for 1 minute at maximum speed (12,000-16,000 x g) and discard the supernatant. |

**Safety Information**

The HiPurA Bone DNA Extraction 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 Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

**Product Use Limitation & Warranty**

HiMedia guarantees the performance of product in the manner described in the product literature. HiPurA Bone DNA Extraction Kit is designed and sold for research and *in vitro* purposes only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. HiPurA Bone DNA Extraction Kit is intended as general-purpose device. No claim or representation is intended for their use to identify any specific organism or for a specific clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of the kit for any particular use, since their performance characteristics have not been validated for any specific organism. The kit may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries. All due care and attention should be exercised in the handling of the product.

**Technical Assistance**

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

**Related Products**

| Related Products | Product code |
|---|---|
| HiPurA™ Forensic Sample Genomic DNA Purification Spin Kit | MB524 |

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