

## MB578 HiPurA® QuickBone DNA Purification Kit (Column Based)

### Kit Contents

Product Code	Reagents provided	MB578	
		20 Preps	50 Preps
DS0075	Bone Extraction Buffer	28 ml	70 ml
DS0077	Wash Solution Concentrate (BW)	16 ml	40 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	1.2 ml	3 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos	50 nos
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos
PW1139	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos

### Intended Use

Recommended for isolation of DNA from human and animal bone samples.

### Introduction

HiPurA® QuickBone DNA Purification Kit (Column Based) provides a fast and easy method for isolation of genomic DNA from bone samples of various species (including forensic samples) using spin column procedure. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting. The DNA purification procedure using the miniprep spin columns comprises of three steps viz. adsorption of DNA to the membrane, removal of residual contaminants and elution of pure genomic DNA. HiMedia's HiElute Miniprep Spin Column (Capped) format allows rapid processing of multiple samples. The columns have a high binding capacity and high quality genomic DNA is obtained from various species. The genomic DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

### HiPurA® QuickBone DNA Purification Kit (Column Based)

This kit simplifies the isolation of DNA from bone with spin column procedure. Once the bone sample is decalcified, it is subjected to lysis by Bone Extraction Buffer. Following lysis, DNA binds to the silica gel membrane of the HiElute Miniprep Spin Column (Capped) to yield purified 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 50 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® QuickBone DNA Purification Kit (Column Based) 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
- Heating block or Water bath at 55°C
- Motar and pestle or freezer mill
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes) (LA1001)
- Molecular Biology Grade Water (Product Code: ML024)

**Storage**

HiPurA® QuickBone DNA Purification Kit (Column Based) can be stored at room temperature (15-25°C) except certain components mentioned on the label for up to 1 year without showing any reduction in performance. The product as supplied is stable at room temperature (15-25°C).

**General Preparation Instructions**

1. **Thoroughly mix reagents**

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

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

Number of Preps	Wash Solution Concentrate (BW)	Ethanol (96-100 %)
20	16 ml	16 ml
50	40 ml	40 ml

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

**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.

**Specimen Handling and Collection**

Collect bone sample in a sterile container and store the sample at room temperature (15-25°C).

## **Types of Specimen**

Clinical samples: bone

Animal tissue: bone

### **Procedure for cleaning of bone samples:**

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

### **Protocol:**

#### **A. Procedure for Decalcification of Bone**

##### **DAY 1**

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 released. However, if you use a freezer mill do not overgrind, as this may fragment the DNA.

3. Weigh 500 mg of bone sample and transfer it to a sterile 2.0 ml Collection tube (provided).

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

4. Add 1.2ml of Bone Extraction Buffer (DS0075) to the sample to decalcify the bone
5. Incubate the sample at 55°C for 16 hours or overnight at approximately 100rpm.

#### **B. Bone DNA Purification Procedure**

##### **DAY 2**

6. Centrifuge the decalcified sample for 2 minutes at 6,500 x g ( $\approx$ 10,000 rpm) at room temperature (15-25°C).

##### **7. Prepare for Binding**

Transfer the supernatant carefully, obtained from step 6, into a new 2.0 ml collection tube (not provided) and add equal volume of ethanol (96- 100%) for preparation of the lysate for binding. Mix thoroughly by vortexing for 5-10 seconds.

##### **8. Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**

Add 650  $\mu$ l of the mixture from step 7, including any precipitate, which may have formed, to the HiElute Miniprep Spin Column (Capped). Centrifuge for 1 minute at 6,500 x g ( $\approx$ 10,000 rpm) at room temperature (15-25°C). Discard the flow-through.

**NOTE:** Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents into the column.

9. Repeat step 8 with the remaining sample. Discard the flow-through liquid.

#### 10. Wash

##### **(Prepare the Wash Solution Concentrate (BW) (DS0077) as indicated in General Preparation Instructions)**

Add 500 µl of diluted Wash Solution (BW) to the HiElute Miniprep Spin Column (Capped) and centrifuge for 1 minute at 6,500 x g (≈10,000 rpm) at room temperature (15-25°C). Discard the flow-through liquid and reuse the same collection tube.

11. Repeat the wash step by adding another 500 µl of diluted Wash Solution (BW) to the HiElute Miniprep Spin Column (Capped) and centrifuge for 1 minute at 6,500 x g (≈10,000 rpm) at room temperature (15-25°C). Discard the flow-through liquid and reuse the same collection tube.
12. Centrifuge the column for 2 minutes at 20,000 x g (≈14,000 rpm) to dry the column membrane to remove the traces of residual ethanol, if observed. Place the column in a new uncapped 2.0 ml collection tube.

#### 13. DNA Elution

Pipette 20-50 µl of the Elution Buffer [10mM Tris-Cl, pH 8.5] (ET) (DS0040) or Molecular Biology Grade Water (ML024) directly onto the centre of the HiElute Miniprep Spin Column (Capped) membrane without spilling to the sides. Incubate for 1 minute at room temperature. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute to elute the DNA.

**NOTE:** To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer, then centrifuge. Elution with volumes less than 50 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. Storing DNA in water can cause acid hydrolysis.

14. Transfer the eluate to a fresh capped 2ml collection tube for longer DNA storage.

**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

Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical 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

Performance of the kit is expected when the kit is used as per the protocol mentioned in the product insert within the expiry period when stored at recommended temperature.

#### Quality Control

Type of Sample	DNA Purity
Human bone	1.6-1.9
Animal bone	1.6-1.9

**References:**

1. Sambrook, J., *et al.* Molecular Cloning: A laboratory Manual, 2<sup>nd</sup> 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® QuickBone DNA Purification Kit (Column Based) 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**

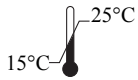
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