

# Purification of DNA from Bone Samples Using Bone DNA Extraction Kit, Custom and DNA IQ™ Chemistry

Promega Corporation

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## 1. Description

This application note provides instructions for use of the Bone DNA Extraction Kit, Custom (Cat.# AX6780) for preprocessing and DNA IQ™ chemistry for purification of DNA from bone and teeth samples. Bone and teeth are traditionally considered difficult sample types due to their unique composition, and typical preprocessing lysis reagents used for casework samples are not effective in efficiently extracting DNA from the calcium matrix. This protocol is based on a Demineralization Buffer protocol developed by the Armed Forces DNA Identification Lab, and has been used in combination with many purification protocols, including organic extraction (1). DNA IQ™ chemistry has been shown to purify DNA from bone lysates, although the preprocessing reagents used were different (2-4).

The new Bone DNA Extraction Kit, Custom contains reagents needed for preprocessing bone samples from pulverized samples. Promega offers both automated (Maxwell®) and manual methods of DNA purification using DNA IQ™ chemistry, and protocols for both methods are included in this Application Note. For additional details regarding manual DNA purification, refer to *DNA IQ™ System—Small Sample Casework Protocol Technical Bulletin #TB296*. For additional details regarding DNA purification using Maxwell® instrumentation, refer to *DNA IQ™ Casework Pro Kit for Maxwell® 16 Technical Manual #TM332* or *Maxwell® FSC DNA IQ™ Casework Kit Technical Manual #TM499*. Purified DNA is suitable for downstream processing applications, such as human DNA quantification using Plexor HY® and PowerQuant® Systems and STR amplification using PowerPlex® STR reagents.

## 2. Before You Start

The quality of an STR profile from a bone sample depends on the type, age and environmental storage condition of the bone. The success of purifying nuclear DNA from bone also depends on DNA integrity. Soil conditions and humidity have a profound effect on DNA quality.

Bone must be preprocessed to efficiently extract DNA from the calcium matrix. The success of the extraction process also depends on the degree of grinding, which can be accomplished by physical grinding or with a drill operated at low speed to reduce heat buildup. The extraction process works most efficiently with finely ground bone in which cells interspersed in the bone matrix are more accessible for lysis.

### 3. Product Components and Storage Conditions

Please contact your Promega representative to order the Bone DNA Extraction Kit, Custom. Contact information available at: [www.promega.com](http://www.promega.com). E-mail: [genetic@promega.com](mailto:genetic@promega.com)

PRODUCT	SIZE
Bone DNA Extraction Kit, Custom	100 Preps

Not For Medical Diagnostic Use.

Includes:

- 50ml Demineralization Buffer, Custom
- 3 x 0.9ml 1-Thioglycerol
- 4ml Proteinase K
- 150ml Lysis Buffer

**Storage Conditions:** Store kit at 15–30°C. Upon receipt, store 1-Thioglycerol (Part# A208B) at 2–10°C.

### 4. Preprocessing Using Demineralization Buffer and Purification Using DNA IQ™ Manual Protocol

For further information, refer to *DNA IQ™ System—Small Sample Casework Protocol Technical Bulletin #TB296*.

**Materials to Be Supplied by the User**

- Bone DNA Extraction Kit, Custom (Cat.# AX6780)
- DNA IQ™ System (Cat.# DC6700 and DC6701)
- PolyAtract® System 1000 Magnetic Separation Stand (Cat.# AS1240) OR MagneSphere® Magnetic Technology Stand (Cat.# Z5332 or Cat.# Z5342)
- 56°C heater/shaker
- 65°C heater/shaker
- 95–100% ethanol
- Isopropyl Alcohol
- Microcentrifuge tubes, 1.5ml
- Aerosol-resistant micropipette tips

**Method**

1. Weigh out 100mg of pulverized bone powder into 1.5ml tubes for processing.
2. Prepare bone lysis cocktail A as follows, allowing for an excess of n+2 samples:

Reagent	Volume for n = 1 sample
Demineralization Buffer	400µl
Proteinase K	40µl
1-Thioglycerol	10µl

Note: Pipette 1-thioglycerol slowly, as it has high viscosity.

3. Add 400µl of bone lysis cocktail A to each 1.5ml tube containing bone powder.
4. Vortex each tube for approximately 10 seconds to mix.

5. Incubate the tubes on a heater/shaker set to 56°C for 2.5 hours, shaking at 1000rpm.
6. Remove the tubes from the heater/shaker and vortex for approximately 10 seconds to mix.
7. Centrifuge the tubes at 13,000 × *g* for 5 minutes.
8. Carefully transfer supernatant to new 1.5ml tubes, taking care not to disturb the pellet. Dispose of tubes containing bone pellets.
9. Prepare bone lysis cocktail B as follows, allowing for an excess of n+2 samples:

Reagent	Volume for n = 1 sample
Lysis Buffer	990µl
1-Thioglycerol	10µl

10. Add 800µl of bone lysis cocktail B to each 1.5ml tube containing lysate from step 8.
11. Vortex each tube for approximately 10 seconds to mix.
12. Vortex the DNA IQ™ Resin to re-suspend the magnetic beads. Add 15µl of resin to each tube, vortex for 3–5 seconds and incubate at room temperature for 5 minutes, vortexing to mix every 2 minutes.
13. Vortex for 3–5 seconds and place in the magnetic stand. Allow the beads to pellet to the side, then gently remove the lysate and discard. Do not disrupt the beads.
14. Add 100µl of Lysis Buffer/1-Thioglycerol solution, vortex for 3–5 seconds, and pellet the beads with magnetic stand. Gently remove and discard the lysis buffer.
15. Dilute the 2X Wash Buffer to 1X as follows:

Reagent	Volume to add for 1X
2X Wash Buffer	30ml
Isopropyl Alcohol	15ml
95-100% Ethanol	15ml

16. Add 100µl of 1X Wash Buffer, vortex for 3–5 seconds and pellet the beads with magnetic stand. Remove and discard the wash buffer. Repeat the washes with 1X Wash Buffer two more times for a total of three washes.
17. With the lid open, air-dry the pellet 5 minutes.
18. Add 50µl of Elution Buffer, close the lid and vortex for 3–5 seconds, and incubate at 65°C for 5 minutes.
19. Vortex for 3–5 seconds, pellet the beads in the magnetic stand and transfer the eluted DNA to a sterile 1.5ml microcentrifuge tube.
20. Store at 4°C for up to 3 weeks. Store at –20°C for long-term storage.

## 5. Preprocessing Using Demineralization Buffer and Purification Using DNA IQ™ Maxwell® Protocol

For further information, refer to *DNA IQ™ Casework Pro Kit for Maxwell® 16 Technical Manual #TM332* or *Maxwell® FSC DNA IQ™ Casework Kit Technical Manual #TM499*.

### Materials to Be Supplied by the User

- Bone DNA Extraction Kit, Custom (Cat.# AX6780)
- Maxwell® DNA IQ™ Casework Pro Kit (Cat.# AS1240), for use with Maxwell® 16 Forensic Instrument (Cat.# AS3060)

### OR

- Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550), for use with Maxwell® FSC Instrument (Cat.# AS4600) or Maxwell® RSC 48 Instrument (Cat.# AS8500)
- 56°C heater/shaker
- Microcentrifuge tubes, 1.5ml
- Aerosol-resistant micropipette tips

### Method

1. Weigh out 100mg of pulverized bone powder into 1.5ml tubes for processing.
2. Prepare bone lysis cocktail A as follows, allowing for an excess of n+2 samples:

Reagent	Volume for n = 1 sample
Demineralization Buffer	400µl
Proteinase K	40µl
1-Thioglycerol	10µl

Note: Pipette 1-thioglycerol slowly, as it has high viscosity.

3. Add 400µl of bone lysis cocktail A to each 1.5ml tube containing bone powder.
4. Vortex each tube for approximately 10 seconds to mix.
5. Incubate the tubes on a heater/shaker set to 56°C for 2.5 hours, shaking at 1000rpm.
6. Remove the tubes from the heater/shaker and vortex for approximately 10 seconds to mix.
7. Centrifuge the tubes at 13,000 × g for 5 minutes.
8. Carefully transfer supernatant to new 1.5ml tubes, taking care not to disturb the pellet. Dispose of tubes containing bone pellets.
9. Prepare bone lysis cocktail B as follows, allowing for an excess of n+2 samples:

Reagent	Volume for n = 1 sample
Lysis Buffer	990µl
1-Thioglycerol	10µl

10. Add 800µl of bone lysis cocktail B to each 1.5ml tube containing lysate from step 8.
11. Vortex each tube for approximately 10 seconds to mix.

## 5.A. DNA Purification Using Maxwell® 16 Instrument

1. Prepare a Maxwell® 16 LEV Cartridge Rack with Maxwell® 16 LEV Cartridges, LEV plungers (Cat.# AS165A) and elution tubes. Add 50µl of elution buffer supplied in the kit (Cat.# AS1240) to the bottom of each elution tube.
2. Transfer the entire volume of eluate from Section 5 step 10 (approximately 1.1ml) to well 1 of the Maxwell® 16 LEV Cartridges and run the Casework protocol.
3. Following the run, the eluted DNA can be stored at 4°C for short-term storage or at –20°C or –70°C for long-term storage.

## 5.B. DNA Purification Using Maxwell® FSC or Maxwell® RSC 48 Instrument

1. Prepare a Maxwell® FSC Deck Tray with Maxwell® FSC Cartridges, FSC plungers (Cat.# AS715A) and elution tubes. Add 50µl of elution buffer supplied in the kit (Cat.# AS1550) to the bottom of each elution tube.
2. Transfer the entire volume of eluate from Section 5 step 10 (approximately 1.1ml) to well 1 of the Maxwell® FSC Cartridge and run the DNA IQ™ Casework protocol.
3. Following the run, the eluted DNA can be stored at 4°C for short-term storage or at –20°C or –70°C for long-term storage.

## 6. Buffer Composition

**Demineralization Buffer:** 0.5M EDTA, pH 8.0, 1% Lauroylsarcosine

## 7. References

1. Loriele, O.M. *et al.* (2007) High efficiency DNA extraction from bone by total demineralization. *Forensic Science International: Genetics*. **1**, 191.
2. Grubb, J.C. *et al.* (2010) Implementation and validation of the Teleshake unit for DNA IQ robotic extraction and development of a large volume DNA IQ method. *J Forensic Sci.* **55**(3).
3. Zoppis, S. *et al.* (2016) Genetic analyses on bone remains with the PowerPlex® Fusion System kit. *Prevention and Research*. **5**(2), 50.
4. Karija Vlahović, M. *et al.* (2012) DNA extraction method from bones using Maxwell® 16. *Leg Med.* **14**(5), 272.

## 8. Ordering Information

Product	Cat. #
Bone DNA Extraction Kit, Custom	AX6780
DNA IQ™ System	DC6700 and DC6701
PolyAtract® System 1000 Magnetic Separation Stand	Z5410
MagneSphere® Magnetic Technology Stand	Z5332 or Z5342
Maxwell® FSC DNA IQ™ Casework Kit	AS1550
Maxwell® FSC Instrument	AS4600
Maxwell® DNA IQ™ Casework Pro Kit	AS1240
Maxwell® Forensic Instrument	AS3060
Maxwell® RSC 48 Instrument	AS8500
FSC Plungers	AS7151
LEV Plungers	AS1651

Please contact your Promega representative to order the Bone DNA Extraction Kit, Custom.  
 Contact information available at: [www.promega.com](http://www.promega.com). E-mail: [genetic@promega.com](mailto:genetic@promega.com)

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