InnoXtract™ Bone DNA Extraction and Purification Kit User Guide

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Kit Contents

100 Reactions (#21124-100)

InnoXtract Bone DNA Binding Buffer (x 2 bottles)	60 mL each
InnoXtract Wash Buffer	57 mL
InnoXtract Bone Digest Buffer	62 mL
InnoXtract Magnetic Bead Suspension (x 2 tubes)	1.1 mL each
InnoXtract Elution Buffer (x 3 tubes)	1.5 mL each

50 Reactions (#21124-50)

InnoXtract Bone DNA Binding Buffer (x 2 bottles)	30 mL each
InnoXtract Wash Buffer	28 mL
InnoXtract Bone Digest Buffer	31 mL
InnoXtract Magnetic Bead Suspension	1.1 mL
InnoXtract Elution Buffer (x 2 tubes)	1.5 mL each

Reagents and Equipment Supplied by User

Fresh 100% ethanol (method optimized with Decon cat# 3916EA)
Proteinase K (pK) 20 mg/mL (method optimized with Invitrogen ProK cat# 25530049)
Vortexing mixer
Magnet stand (method optimized on a ThermoFisher DynaMag™-2)

Prior to Initial Use

The Binding and Wash Buffers are shipped as concentrates. If precipitate is present in either solution, incubate solution at 37°C for 30 minutes. 100% EtOH at room temperature must be added to both solutions before the first use.

For 100 reaction kit (# 21124-100):

- Add 20 mL of fresh 100% ethanol to each bottle of Bone DNA Binding Buffer and mix by inverting gently. Indicate on the bottle that ethanol was added and include the date it was added on the bottle.
- Add 53 mL of fresh 100% ethanol to the bottle of Wash Buffer and mix by inverting gently. Indicate on the bottle that ethanol was added and include the date it was added on the bottle.
- Prepare fresh 80% ethanol solution with DI water (18 Mega Ohm) prior to each extraction.

For 50 reaction kit (# 21124-50):

- Add 10 mL of fresh 100% ethanol to each bottle of Bone DNA Binding Buffer and mix by inverting gently. Indicate on the bottle that ethanol was added and include the date it was added on the bottle.
- Add 26 mL of fresh 100% ethanol to the bottle of Wash Buffer and mix by inverting gently. Indicate on the bottle that ethanol was added and include the date it was added on the bottle.
- Prepare fresh 80% ethanol solution with DI water (18 Mega Ohm) prior to each extraction.

Storage Conditions

Binding Buffer and Wash Buffer

- Once EtOH is added, these buffers are stable for one year if stored at 4°C. Bottle must be tightly closed for long term storage.

Bone Digest Buffer

- Bone digest buffer should be kept at room temperature, never refrigerated. Bone digest buffer is stable for 1 year at room temperature.

DNA Extraction Protocol

NOTE: Allow all reagents to come to room temperature before use.

A. Bone Powder Digestion

1. Transfer 40 mg of bone powder into a 1.5 mL microcentrifuge tube with Bone Digest Buffer and 20 mg/mL Proteinase K (see table below for volume of each). Make sure to fully submerge bone powder.
   *Important: InnoGenomics has validated this method using 40 mg of bone powder. Increasing the bone powder may cause precipitation during the binding steps.

Volume of Reagents Necessary for Bone Digestion		
Bone Digest Buffer (µL)	**Volume of 20 mg/mL pK (µL)**	**Total Volume (µL)**
563	37	600

2. Incubate the sample(s) at 56°C for 5 hours (or overnight) at 1100 rpm.
3. After incubation, centrifuge at max speed (10,000 xg or higher) for 5 minutes. If the lysate is still cloudy or colored, centrifuge at max speed for an additional 5 minutes. Transfer clear lysate to a new 1.5 mL microcentrifuge tube.
   *Important: Transfer the clear lysate (clear liquid) and avoid transferring any undigested bone powder. Undigested bone powder reduces DNA binding efficiency of magnetic beads.

B. Two Step DNA Binding

1. Place the lysate containing tube(s) on ice for 5 minutes to cool the tube(s) to room temperature, if necessary.
   *Efficiency of magnetic beads' DNA binding decreases at higher temperature. It is crucial that the sample solution is at room temperature before proceeding.
2. First DNA binding step: Transfer 300 µL of the clear lysate (from Section A, step 3) to a new 1.5 mL microcentrifuge tube.
3. Add 750 µL of **Bone DNA Binding Buffer*.**

*Caution: If the Bone DNA Binding Buffer has been stored at 4°C, equilibrate the buffer to room temperature before use. Cold buffer may cause cloudy solution (or salt precipitation).

4. Add 20 µL of **Magnetic Bead Suspension.**

   *Important: Mix beads well prior to adding. There should be no visible sedimentation at the bottom of the solution after mixing. Beads will settle quickly, ensure the Magnetic Bead Suspension is well mixed before adding to each sample. Failure to do so may result in inconsistent yields. It is recommended to aliquot the total amount of Magnetic Bead Suspension needed at one time into a separate tube before adding to samples.

5. Vortex tube(s) for 10 minutes.

   *To obtain high yields, ensure that the solution is mixed vigorously in tube(s). A vortexing mixer with a tube-holder that allows for walk-away mixing will make this easier. If this is not available, the samples can be vortexed for 10 seconds, placed in a thermomixer at 25°C for 10 minutes at 1400 rpm, then vortexed for 10 seconds.

6. Centrifuge briefly.

   * Centrifuge steps are needed when vortexing to resuspend beads. Only a brief spin is recommended to remove solution from tube(s) lid.

7. Place tube(s) into a magnetic stand for 2 to 5 minutes, or until solution clears.
8. While keeping the tube(s) on the magnetic stand, remove the supernatant. Be careful not to remove the magnetic particles.
9. Keep tube(s) on magnetic stand for 1 minute and remove any residual supernatant.
10. **Second DNA binding step**: Transfer the remaining lysate (< 300 µL) to the same magnetic bead containing tube and add 750 µL of **Bone DNA Binding Buffer.**
11. Repeat steps 5-9.

C. First Wash

1. To the tube(s) containing magnetic bead add 500 µL of **Wash Buffer**.
2. Resuspend beads by vortexing for 10 seconds or pipetting up and down 6 times.
3. Centrifuge tube(s) briefly.
4. Keep tube(s) on the magnet stand for 10-30 seconds or until the solution is clear.
5. Remove as much buffer as possible using a 1000 µL pipette or a transfer pipette.
6. Tap magnet stand on the bench 5 times and remove the remaining wash buffer with a 200 µL pipette.
7. Repeat Step 1-6 of this first wash.

D. Second Wash

1. Add 500 µL of **80% EtOH.**
2. Resuspend beads by vortexing for 10 seconds or pipetting up and down 6 times.
3. Centrifuge tube(s) briefly.
4. Place tube(s) on the magnetic stand for 10-30 seconds or until solution clears.
5. Remove as much EtOH as possible using a 1000 µL pipette or a transfer pipette.
6. Add 500 µL of **80% EtOH.**
7. Resuspend beads by vortexing for 10 seconds or pipetting up and down 6 times.
8. Centrifuge tube(s) briefly.
9. Place tube(s) on the magnetic stand for 10-30 seconds or until solution clears.
10. Remove as much EtOH as possible using a 1000 µL pipette or a transfer pipette and leave cap open.
11. Tap the magnetic stand with tube(s) on the bench 5 times.
12. Remove the remaining EtOH with a 200 µL pipette.
13. Leave the tube(s) open on the magnetic stand for 2 minutes and then tap the tube(s) on the bench 5 times and remove any remaining EtOH with a 20 µL pipette.
14. Allow the magnetic particles to dry for an additional 1-3 minutes.

    *Be careful to not over dry or the beads will stick to tube(s), resulting in low yield.

E. Elution Step

1. Transfer the microtube(s) to a non-magnetic rack, add 40 µL of **InnoXtract Elution Buffer** and resuspend the beads.
2. Vortex the tube(s) for 5 minutes.

   *To obtain high yields, ensure that the solution is mixed vigorously in tube(s). A vortexing mixer with a tube-holder that allows for walk-away mixing will make this easier. If this is not available, the samples can be vortexed for 10 seconds, placed in a thermomixer at 25°C for 5 minutes at 1400 rpm, then vortexed for 10 seconds.
3. Centrifuge tube(s) briefly.
4. Place tube(s) on the magnetic rack for 10-30 seconds.
5. Transfer eluate into a new 1.5 mL tube.

6. Incubate sample(s) at 50°C for 5 minutes while leaving tube cap open.

   *This step is designed to remove any remaining ethanol that may inhibit PCR reactions. If sample evaporates beyond desired amount, add nuclease-free water to increase total volume.

NOTE: During the development of products for forensic DNA analysis, InnoGenomics Technologies performs developmental validation studies. However, it is the responsibility of the customer laboratory to perform its own analysis and internal validation studies, and develop its own standard operating procedures and interpretation guidelines, to ensure that the products and services it obtains from InnoGenomics Technologies satisfy or will satisfy the applicable guidelines used by the forensic community and are fit for the customer laboratory's human identification applications.

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