【Product Name】HiPure Bone DNA Kit

【Product specifications】20 Preps/Kit, 50 Preps/Kit, 250 Preps/Kit

【Intended Use】

This product provides fast and easy method for purification of high purity DNA from bone samples. The obtained DNA can be directly used for PCR and STR detection down stream application.

【Kit Contents】

Cat.No.	D312401	D312402	D312403
Purification Times	20 Preps	50 Preps	250 Preps
HiPure DNA Mini Columns I	20	50	250
2ml Collection Tubes	20	50	250
Buffer BGL	20 ml	40 ml	180 ml
Buffer GXP	15 ml	30 ml	150 ml
Buffer GW1*	6 ml	13 ml	53 ml
Buffer GW2*	6 ml	20 ml	2 x 50 ml
DTT Powder	235 mg	235 mg	2 x 235 mg
Proteinase K	24 mg	48 mg	240 mg
Protease Dissolve Buffer	1.8 ml	5 ml	15 ml
Elution Buffer	5 ml	15 ml	60 ml

【Storage conditions and Validity】

This product can be stored at room temperature (15~25°C) for 18 months. Proteinase K/DTT dry powder can be transported and stored at room temperature. For long-term storage (>6 months), it is recommended to store at -20~8°C. Dissolved Proteinase K should be stored at -20~8°C. The dissolved DTT should be stored at -20°C.

【Preparation before Use】

- Add 1.2ml (20Preps) or 2.4ml (50 Preps), or 12ml (250Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8°C after dissolve.
- Add 1.5ml Elution Buffer to DTT dry powder, vortex to mix throughly. Use or store at -20°C.
- Add 24ml (20Preps) or 80ml (50Preps) or 2x200ml (250Preps) absolute ethanol to the bottle of Buffer GW2 and store at room temperature.
- Add 8ml (20Preps) or 17ml (50Preps) or 67ml (250Preps) absolute ethanol to the bottle of Buffer GW1 and store at room temperature.

Bond grinding

The quality of STR maps from bone samples depends on the type of bone, age, and environmental storage conditions. Soil conditions and moisture have a profound effect on DNA quality. The success of the extraction process depends on the degree of grinding, which can be achieved by physical grinding or with a bit operated at a low speed to reduce heat build-up. The extraction process works best for fine-ground bone meal, where cells scattered throughout the bone matrix are easier to digest.

Bone meal grinder: Pre-cool teeth or bones with liquid nitrogen, and pre-cool bone meal grinders with liquid nitrogen. Transfer the sample to the bone meal grinder, beat it hardly with a hammer several times, pre-cool the grinder with liquid nitrogen, beat it several times until the sample forms a partial fine powder and small bone fragments, transfer the sample to the container, gently shake, and continue grinding the large sample into powder. Gently oscillate in the container to pick out the fine powder for extraction process.

Bead mill: Please refer to the protocol of bead mill.

Protocol

1. **Transfer 100-200 mg bone powder into a new 2.0ml centrifuge tube. Add 600μl Buffer BGL, 6μl 1M DTT, and 40μl Proteinase K, mixing upside down several times**. Mix by shaking at 1000-1500rmp at 55°C water bath for 3~24 hours.

   Note: The digestion of small bone powder for 3 hours can also obtain enough DNA. If time available, it can extend the digestion time to 24 hours.
2. Centrifuge at 13,000 x g for 5 minutes at room temperature.
3. **Transfer 500μl of supernatant into a new 2.0ml centrifuge tube. Add 500μl Buffer GXP and 250μl absolute ethanol to the tube and mix upside down for10-15 times**.
4. **Insert HiPure DNA Mini Column I into the 2ml collection tube. Transfer 600μl mixture into the column.** Centrifuge at 12,000 x g for 1 min.
5. **Discard the filtrate and put the column back in the collection tube. Transfer the remaining mixture to the column.** Centrifuge at 12,000 x g for 1 min.
6. **Discard the filtrate and put the column back in the collection tube. Add 500μl Buffer GW1 to the column.** Centrifuge at 12,000 x g for 1 min.
7. **Discard the filtrate and put the column back in the collection tube. Add 500μl Buffer GW2 to the column**. Centrifuge at 12,000 x g for 1 min.
8. Repeat step 7 once.
9. Discard the filtrate and put the column back in the collection tube. Centrifuge the empty column at 12,000 × g for 2 minute at room temperature to dry the column matrix.
10. Open the lid and incubate at room temperature for 10~15 min to dry the column..
11. **Install the column in a new 1.5ml centrifuge tube. Add 20~50μl Elution Buffer (preheated to 55℃) directly into the center o the column membrane.** Let sit at room temperature for 3 min. Centrifuge at 12,000 × g for 1 min.
12. Discard the column, store the DNA at 2-8°C. For long-term storage, store it at -20℃.

Troubleshooting Guide

1. Column blocked

- **Too much sample:** Reduce the sample amount, for samples rich in nucleic acid such as liver, spleen, lung, dosage, it should not exceed 10mg.
- **Sample not digested throughly:** Grind and homogenize the sample by liquid nitrogen or glass homogenizer to improve bone digestion efficiency. Extend Proteinase K digestion time or overnight digestion.
- **Insoluble substances in digestive buffer:** If there are still significant particles after digestion, centrifuge at 12,000 x g for 3 min to remove the undigested material.

2. Low or no recovery

- **Refer to the blocked part of the column**
- **Insufficient sample digestion:** Extend the digestion time to allow the sample to fully digest, and homogenize the sample with a glass homogenizer.
- **Buffer GW1/GW2 did not contain ethanol:** Ethanol must be added to Buffer GW1/GW2 before used. Repeat procedure with correctly prepare Buffer
- **Inadequate elution**: The elution buffer needs to be added to the center of the membrane. Increase the elution volume or elute more times.

3. Insufficient DNA purity

- **Too much sample:**: Reduce the sample amount.