



## Genorise<sup>®</sup> Bone DNA Extraction Kit 1000

This kit is to isolate total DNA including genomic and mitochondrial DNA from 20 mg bone and is for 1000 applications. This kit can significantly improve quantity and quality of DNA and is much more cost-effective than the similar products.

### Materials provided in the kit:

120 ml 5 x Lysis Solution  
220 ml Protein Precipitation Solution  
220 ml DNA Hydration Solution  
1.5 ml 10 x DNA Sample Loading Buffer

### Materials required but not provided in the kit:

Proteinase K, prepare at 20 mg/ml and store at -20°C  
Isopropanol (2-propanol)/Ethanol

## Protocol

### Cell Lysis

1. Add 0.4 ml DNA water and 0.1 ml of 5 x Lysis Solution to 20 mg grounded bone in a 1.7 ml microtube, vortex for 20 sec, and add 3 µl Proteinase K solution (20 mg/ml), vortex for 20 sec.
2. Incubate at 55°C for 1 hr or overnight until the cell lysate becomes completely clear (disrupted).

### Protein precipitation

1. Cool sample to room temperature by placing on ice for 1 min.
2. Add 0.2 ml of Protein Precipitation Solution to the lysate.
3. Vortex samples at high speed for 20 sec and place sample into an ice bath for 5 min.
4. Centrifuge at 15,000 x g for 5 min.

### DNA Precipitation

1. Pour the supernatant containing DNA into a new 1.7 ml microcentrifuge tube.
2. Centrifuge at 15,000 x g for 5 min.
3. Pour the supernatant to a new microcentrifuge tube; repeat step 1 and 2 until no pellet is seen.
4. Pour the supernatant containing DNA into a new 1.5 ml microcentrifuge tube containing 0.5 ml 100% isopropanol (2-propanol).
5. Mix the samples by inverting gently 50 times and incubate at room temperature for 10 min.
6. Centrifuge at 13,000 x g for 5 min.
7. Pour off the supernatant and drain the tube briefly on clean absorbent paper. Add 1 ml of 70% ethanol and invert the tube several times to wash the DNA pellet.
8. Centrifuge at 13,000 x g for 5 min, carefully pour off the ethanol and do not lose the DNA pellet.
9. Invert and drain the tube on clean absorbent paper, completely remove the remaining liquid by a pipette, and finally allow air dry for 5 min.

### DNA Hydration

1. Add 50 µl DNA Hydration Solution.
2. Resuspend the DNA by a pipette for 5 times and rehydrate the DNA by incubation for 10 min at room temperature.
3. Vortex briefly and pulse spin before use, and store at -20°C.

### Note

1. Following DNA precipitation, if you see a big pellet with a color other than white, we recommend you repeat this protocol with a start volume of 0.5 ml of DNA isolate and 1 hr cell lysis.
2. Do not air dry DNA pellet more than 5 min.