Genorise® Semen DNA Extraction Kit 1000

This kit is to isolate total DNA including genomic and mitochondrial DNA from 0.3 ml of semen and is for 1000 applications. If a smaller or larger sample volume is employed, the reagent quantity should be proportionally decreased or increased. This kit can significantly improve quality and quantity of DNA and is more cost-effective than the similar products.

Materials provided in the kit:
120 ml 5 x Lysis Solution
300 ml Protein Precipitation Solution
200 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 -20ºC
Isopropanol (2-propanol)/Ethanol

Protocol

Cell Lysis

1. Add 0.4 ml DNA water and 0.1 ml of 5 x Lysis Solution to 0.2 ml fresh semen in a 1.5 ml microcentrifute tube, vortex for 20 sec, and add 3 µl Proteinase K solution (20 mg/ml), vortex for 20 sec.
2. Incubate at 55°C overnight until cell lysate becomes completely clear (cells disrupted).

Protein precipitation

1. Cool sample to room temperature by placing on ice for 1 min.
2. Add 0.3 ml of Protein Precipitation Solution to the lysate.
3. Vortex samples at high speed for 20 sec.
4. Place sample into an ice bath for 5 min.
5. Centrifuge at 15,000 x g for 5 min.

DNA Precipitation

1. Pour the supernatant containing DNA into a new 1.5 ml microcentrifute 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 microcentrifute tube containing 0.7 ml 100% isopropanol.
5. Mix the samples by inverting gently 50 times and incubate at room temperature for 5 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 tube and drain the tube on clean absorbent paper, remove the remaining liquid by a pipette, and finally allow airing dry for 5 min.

DNA Hydration

1. Add 50 µl DNA Hydration Solution.
2. Resuspend the DNA pellet 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.

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Note

1. Following DNA precipitation, if you see a big pellet with a color other than white, we recommend you repeat this protocol with 1 hr cell lysis.
2. Air drying of DNA pellet should not exceed 5 min.