Genorise® Semen DNA Extraction Kit 500

This kit is to isolate total DNA including genomic and mitochondrial DNA from 0.3 ml of semen and is for 500 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 much more cost-effective than the similar products.

Materials provided in the kit:
60 ml 5 x Lysis Solution
150 ml Protein Precipitation Solution
100 ml DNA Hydration Solution
1.0 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. Take 0.2 ml fresh semen to a 1.5 ml microcentrifute tube
2. Dilute 5 x Lysis Buffer 5 times to 1 x by DNA water and add 0.5 ml of diluted 1 x Lysis Solution and 3 µl Proteinase K solution (20 mg/ml), vortex for 20 sec.
3. 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 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 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.