FavorPrep™ Tissue Genomic DNA Extraction Mini Kit
Spical protocol for human sperm DNA extraction v.1212

(Cat.: FATGK001, FATGK001-1)
(For Research Use Only)

Kit Contents:

FATG1 Buffer	**15 ml**
FATG2 Buffer	**15 ml**
W1 Buffer * (concentrated)	**30 ml**
Wash Buffer * (concentrated)	**12.5 ml**
Elution Buffer	**10 ml**
Proteinase K	**11 mg**
FATG Column	**50 pcs**
Collection Tube	**100 Pcs**
Elution Tube	**50 Pcs**

*Add ethanol (96- 100 %) to W1 Buffer and Wash Buffer before use.

Additional Material Provided By User:

NaCl (0.9%)
DTT (1M)
RNase A (50mg/ml)
Ethanol (96%~100%)
water baths to 56 °C

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Add 1.1 ml of sterile ddH_2O to Proteinase K tube to make a **10 mg/ml** stock solution. Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4 °C.**
3. Add required volumn of ethanol (96- 100 %) to W1 Buffer and Wash Buffer when first open.
4. Prepare a water baths to 56 °C before the operation.

Sperm DNA Extraction protocol

Please Read Important Notes Before Starting The Following steps.

1. Add 1.5 ml of 0.9% NaCl to a microcentrifuge tube. (not provided)

2. Add 10 µl of sperm sample and invert the tube 5 times.

3. Centrifuge the tube at 14,000 rpm for 2 min.

4. Discard the supernatant. ***Note!*** Do not disrupt the cell pellet.

5. Add 200 µl of **FATG1 Buffer** and resuspend the cell by pipetting.

6. **(Optional)** Add 5 µl of **RNase A (50mg/ml) to the sample mixture** mix well by pipetting and incubate at room temperature for 5 min**.**

7. Add 20 µl of **Proteinase K (10 mg/ml)** to the sample mixture and mix well by pipetting. Incubate the sample mixture at 56 °C for 30 min, vortex every 10 min during the incubation.

8. Add 10 µl of **DTT (1M)** to the sample mixture and mix well by pipetting. Incubate the sample mixture at 56 °C for 30~60 min until the sample mixture is clear, vortex every 10 min during the incubation.

9. Briefly spin the tube to remove drops from the inside of the lid.

10. Add 200 µl of FATG2 Buffer to the sample mixture, mix well by piptting, and incubate at 70 °C for 10 min.

11. Briefly spin the tube to remove drops from the inside of the lid.

12.Add 200 µl of ethanol (96~100%) to the sample mixture, mix well by plus-vortexing.

13. Place a **FATG Column** in **Collection Tube**. Transfer the sample mixture (including any precipitate) carefully to **FATG Column**. Centrifuge at 8,000 rpm for 1 min **then place the FATG Column to a new Collection Tube.**

14. Wash the **FATG Column** with 500 µl of **W1 Buffer** by centrifuge at 14,000 rpm for 1 min. Discard the flow-through, place the FATG Column back to the Collection Tube.
--Make sure that ethanol has been added into W1 Buffer when first open.

15. Wash the **FATG Column** with 750 µl of **Wash Buffer** by centrifuge at 14,000 rpm for 1 min. Discard the flow-through, place the FATG Column back to the Collection Tube.
--Make sure that ethanol has been added into Wash Buffer when first open.

16. Centrifuge at 14,000 rpm for an additional 3 min to dry the column.
Important Step! The residual liquid can affect the quality of DNA and inhibit subsequent enzymatic reactions.

17. Place the **FATG Column** to a **Elution Tube**.

18. Add 50 ~100 µl of Elution Buffer or ddH_2O to the membrane center of the **FATG Column**.
Stand **FATG Column** for 3 min.
Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.

19. Centrifuge at 14,000 rpm for 2 min to elute total DNA.

20. Store total DNA at 4°C or -20°C.