(For Research Use Only)

FavorPrep™ Tissue Genomic DNA Extraction Mini Kit

Cat. No., FATGK 001, 001-1, 001-2

Special protocol for milk DNA extraction

26-Nov-2019

Please Read Important Notes of FATGK User Manual Before Starting The Following steps.

Hint: • Set dry or water baths: 37 °C for step 2 and 60 °C for step 3.
• Preheat Elution Buffer to 65 °C for elution step

1. Transfer **up to 1 ml of milk sample** to a microcentrifuge tube (not provided) and centrifuge at full speed (~18,000 x g) for 3 minutes. Discard the supernatant including the creamy floats on the top layer after centrifugation and use a paper towel or a cotton swap to remove any white remains on the tube wall.
2. Add **450 µl of Elution Buffer and Lysozyme (not provided) to final 0.5 mg/ml.** Resuspend the pellet and mix the mixture by vortexing. Incubate at **37°C for 30 min**.
3. Centrifuge the sample at full step for 3 min and discard the saupanatant completely by pipetting.
4. Add **450 µl of FATG1 and 45 µl of Proteinase K solution (10 mg/ml).** Resuspend the pellet and mix the mixture by vortexing. Incubate at **60 °C for 30 min**.
5. Add **450 µl of FATG2 and** mix the sample mixture by vortexing. Incubate at **70 °C for 15 min**.
6. Add **450 µl ethanol (96~100%)** to the sample mixture. Mix thoroughly by pulse-vortexing for 5 sec**.**
7. Place a FATG Column to a Collection Tube. Transfer **up to 750 µl of** the sample mixture to the FATG Column and centrifuge at full speed for 1 min. Discard the flow-through and place the FATG Column back to the Collection Tube.
8. Repeat Step 5 for the rest of the sample mixture.
9. Discard the Collection Tube then place the FATG Column to a new Collection Tube.
10. **Go to Protocol " Isolation of DNA from Animal Tissue" and starting from step 10**

Protocol: Isolation of DNA from Animal Tissue

Please Read Important Notes Before Starting Following Steps.

1. Cut up to 25 mg tissue sample to a microcentrifuge tube (not provided). Use provided Micropestle to grind the tissue sample. Or you can grind the tissue sample in liquid nitrogen with mortar and pestle then transfer the powder to a microcentrifuge tube.
   --- If DNA is prepared from spleen tissue, no more than 10 mg should be used.
2. Add 200 µl FATG1 Buffer and mix well by Micropestle or pipette tip.
3. Add 20 µl Proteinase K (10mg/ml) to the sample mixture. Mix thoroughly by vortexing.
4. **Incubate at 60 °C until the tissue is lysed completely (1~3 h).** Vrotex occasionally during incubation.
   --- Sample can be incubated overnight as well for complete lysis.
5. **(Optional)** If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A (not provided). Mix thoroughly by vortexing and incubate at room temperature for 2 min.
6. Add 200 µl FATG2 Buffer to the sample mixture, mix thoroughly by pulse-vortexing and **incubate at 70 °C for 10 min.**
7. Add 200 µl ethanol (96-100%) to the sample mixture. Mix thoroughly by pulse-vortexing.
8. Briefly spin the tube to remove drops from the inside of the lid.
9. Place a FATG Mini Column in a Collection Tube. Transfer the mixture (including any precipitate) carefully to the FATG Mini Column. Centrifuge at full speed (~18,000 x g) for 1 min **then place the FATG Mini Column to a new Collection Tube.**
10. Add 400 µl W1 Buffer to the FATG Mini Column. Centrifuge at full speed for 1 min then discard flow-through.
    ---Make sure that ethanol has been added into W1 Buffer when first open.
11. Add 750 µl Wash Buffer to the FATG Mini Column. Centrifuge at full speed for 1 min then discard flow-through.
    ---Make sure that ethanol has been added into Wash Buffer when first open.
12. Centrifuge at full speed for an additional 3 min to dry the column.
    --- Important Step! This step will remove the residual liquid.
13. Add 100 µl of preheated Elution Buffer or ddH_2O (pH 7.5-9.0) to the membrane of the FATG Mini Column. Stand the FATG Mini 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.
    --- If less sample to be used, reduce the elution volume to 50 µl to increase DNA concentration and do not elute the DNA using less than suggested volume (50 µl). It will lower the final yield.
14. Centrifuge at full speed for 2 min to elute DNA.