Tissue Genomic DNA Extraction mini Kit

(Cat: FATGK001)
(For research use only)

Special protocol for extraction of DNA from milk sample

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Check FATG1 Buffer before use, Warm fATG1 Buffer at 60°C for 10 minutes if any precipitate formd.
3. Add indicated volume of ethanol (96-100%) to Wash Buffer before use.
4. Prepare a heating block or a water bath to 60 °C.
5. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.
6. Preheat Elution Buffer or ddH2O to 65°C for elution step.
7. If the sample is containing few DNA and getting more yield is needed, please preheat the Elution Buffer. We recommend 65°C as the heating temperature.

Additional requirement:

Lysozyme
Benchtop microcentrifuge
Vortex or bead beater equipment
1.5 ml microcentrifuge tubes
Heating block or a water bath to 60°C and 70°C
96~100 % ethanol

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 4.
• Preheat Elution Buffer to 65 °C for elution step

1A. 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.

1B. Transfer **up to 20 mg of powder milk sample** to a microcentrifuge tube (not provided). Add 1 ml of ddH2O and mix well by vertexing. 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 vertexing. 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.
8. 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.
9. Repeat Step 8 for the rest of the sample mixture.
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 650 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 40~100 µl of preheated Elution Buffer or ddH2O (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 40 µl to increase DNA concentration and do not elute the DNA using less than suggested volume (40 µl). It will lower the final yield.
14. Centrifuge at full speed for 2 min to elute DNA.