User manual

FavorPrep™ Tissue Genomic DNA Extraction MicroElute Kit

(For Research Use Only)

Kit Contents:

Cat. No:	FATGM 000B (4 preps)	FATGM 001B (50 preps)	FATGM 001-1B (100 preps)
FATG1 Buffer	1.5 ml	15 ml	30 ml
FATG2 Buffer	1.5 ml	15 ml	30 ml
W1 Buffer *	1.3 ml	22 ml	44 ml
Wash Buffer **	1.0 ml	10 ml	20 ml
Elution Buffer	1.5 ml	15 ml	30 ml
Proteinase K	1.0 mg	11 mg	11mg x 2
TGM Columns (blister packaging)	4 pcs	10 pcs x 5	10 pcs x 10
Elution Tubes	4 pcs	50 pcs	100 pcs
Micropestles	4 pcs	50 pcs	100 pcs
Collection Tubes	4 pcs	50 pcs	100 pcs

Storage:

1. Kit components except Proteinase K should be stored at room temperature (15 - 25 °C) .
2. Proteinase K should be stored at -20 °C upon receipt.
3. After dissolving, Proteinase K should be stored at 4~8 °C.
4. Store the TGM Columns to 4 ~ 8 ℃ upon receipt

Quality Control:

The quality of FavorPrep™ Tissue Genomic DNA Extraction MicroElute Kit is tested on a lot-to-lot basis according to ISO quality management system.

Product Specification:

Format/ Principle: spin column/ silica membrane/ chaotropic salt
Sample size: up to 10 mg of animal tissue
Operation time: < 60 min Binding capacity: 10 μg/ column
Column applicability: centrifugation

Materials and equipments provided by the user

For All Protocol:

- Pipets, pipet tips and centrifuge tubes (1.5 ml), sterile
- 96 ~100 % ethanol (for preparation of W1 Buffer and Wash Buffer).
- A micro-centrifugator is required, capable of 18,000 X g, with a rotor for 1.5 ml micro-centrifuge tube.

Working Buffer Preparations:

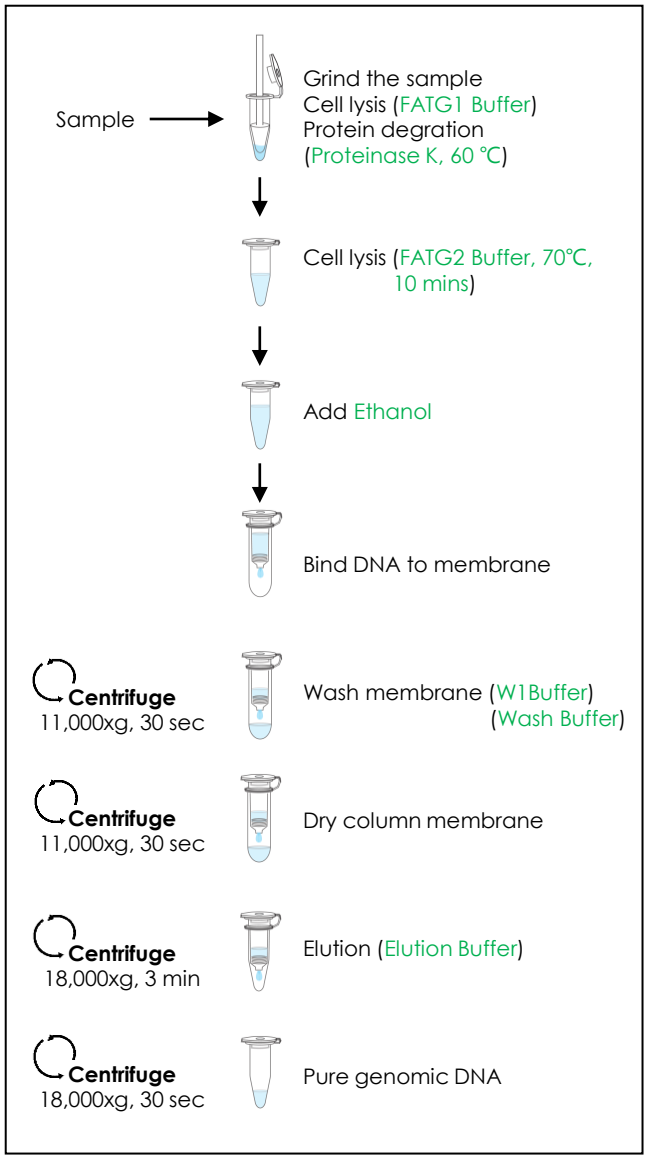
1. Preparation of 10 mg/ ml proteinase solution:
   Add 1.1 ml of ddH_2O to lyophilized proteinase K tube (11mg) and dissolve well Briefly spin the tube. **Store the proteinase K solution at 4~8 °C.**
2. Preparations of W1 Buffer and Wash Buffer:
   Add required ethanol (96~100%) as the table below indicated.
   Store W1 Buffer and Wash Buffers (ethanol added) at 15~25 °C.

Cat. No.	FATGM 000	FATGM 001	FATGM 001-1
Ethanol for W1 Buffer *	0.5 ml	8ml	16ml
Ethanol for Wash Buffer **	4ml	40ml	80ml

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves andlab coat when handling these buffers.
2. Prepare two dry baths or two water baths before the operation:one to 60 °C for step 4 and the other to 70 °C for step 7.
3. Preheat the Elution Buffer to 70 °C for step 16.
4. If precipitates have formed in FATG1 Buffer warm the buffer in 37°C water bath to dissolve precipitates
5. Add ddH_2O to lypohilized proteinase K to make 10 mg/ ml solution when first use. ***see, Working Buffer Preparation***
6. Add ethanol (96~100%) to W1 Buffer and Wash Buffer when first use. ***see, Working Buffer Preparation***
7. Ensure that the centrifugation speed is according to the description of the individual step.

Brief procedure:



Safety Information:

CAUTION: FATG2 Buffers and W1 Buffer contain guanidinium salts which can form highly reactive compounds when combined with bleach. **DO NOT add bleach or acidic solutions directly to the preparation waste.**

Genernal Protocol:

Please Read Important Notes Before Starting The Following steps.

1. Cut the tissue sample up to 10 mg and transfer to a micro-centrifuge tube (not provided). Use provided Micropestle to grind the tissue sample into mud.

2. Add 200 µl of FATG1 Buffer and mix well by micropestle or pipette tip.

3. Add 20 µl of Proteinase K (10mg/ml) to the sample mixture. Mix thoroughly by vortexing.

4. **Incubate at 60 ˚C until the tissue is lysed completely.** Vortex every 10~15 min during incubation.

5. Centrifuge the tube at 4,500 x g for 1 min to pellet the debris and transfer the clarified lysate to a new microcentrifuge tube.

6. **(Optional)** If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A (not provided) and incubate for 2 min at room temperature.

7. Add 200 µl of FATG2 Buffer to the sample mixture. Mix thoroughly by pulse-vortexing and **incubate at 70 ˚C for 10 min.**

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

9. Add 200 µl of ethanol (96-100%) to the sample. Mix thoroughly by pulse-vortexing.

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

11. Place a TGM Column in a Collection Tube. Transfer the mixture carefully to TGM Column. Centrifuge at 11,000 x g for 30 sec **then place TGM Column to a new Collection Tube.**

12. Wash the TGM Column with 400 µl of W1 Buffer by centrifuging at 11,000 x g for 30 sec. Discard the flow-through then place the TGM Column back to the Collection Tube.
    -- Make sure that ethanol has been added to W1 Buffer when first open.

13. Wash the TGM Column with 750 µl of Wash Buffer by centrifuging at 11,000 x g for 30 sec. Discard the flow-through then place the TGM Column back to the Collection Tube.
    --Make sure that ethanol has been added to Wash Buffer when first open.

13. Centrifuge at ~18,000 x g for an additional 3 min to dry the membrane of the TGM Column.
    Important Step! The buffer residue should be completely removed by this step to prevent inhibition on the downstream enzyme reaction.

15. Place the TGM Column to Elution Tube.

16. Add ≥ 10 µl of preheated Elution Buffer or ddH_2O (pH 7.5-9.0) to the membrane center of the TGM Column. Stand the TGM Column for 3 min.
    Important Step! For efficient elution, ensure that the elution solution is dispensed at the center of the membrane and is completely absorbed by the membrane.
    -- **Note !** 10 µl is the ideal elution volume for reaching a high DNA concentration. Do not use the elution buffer less than 10 µl, that it will lower the final yield.
    -- **Note: The average eluate volume is 8 µl from 10 µl elution volume.**

18. Centrifuge at ~18,000 x g for 1 min to elute total DNA.

19. Store total DNA at -20˚C.

• **Problem**/ ☐ Possible reasons/ → Solutions

• **Low or no yield of genomic DNA**
  ☐ To much amount of sample was used
  →Reduce the sample volume
  ☐ Poor cell lysis because of insufficient Proteinase K activity
  → Use a fresh or well-stored Proteinase K stock solution
  →Do not add Proteinase K into FATG2 Buffer directly.
  ☐ Poor cell lysis because of insufficient mixing with FATG2 buffer.
  → Mix the sample and FATG2 Buffer immediately and thoroughly by pulse-vortexing.
  ☐ Poor cell lysis because of insufficient incubation time
  → Extend incubation time and make sure that no residual particle remain.

• **Insufficient binding of DNA to membrane of column**
  ☐ Ethanol is not added into sample lysate before DNA binding
  → Make sure that the correct volumes of ethanol (96- 100 %) is added into the sample lysate before binding.
  ☐ Ethanol and sample lysate did not mix well before DNA binding
  → Make sure that Ethanol and sample lysate have been mixed completely before DNA binding

• **Incorrect preparation of W1 Buffer or Wash Buffer**
  ☐ The percentage of ethanol is not correct in W1 or Wash Buffer
  → Make sure that the correct volumes of ethanol (96- 100 %) is added into Wash Buffer and W1 Buffer when first open.

• **Elution of genomic DNA is not efficient**
  ☐ pH of water (ddH_2O) for elution is acidic
  → Make sure the pH of ddH_2O is between 7.5-9.0.
  → Use Elution Buffer (provided) for elution.
  ☐ Elution Buffer or ddH_2O is not completely absorbed by membrane
  → After Elution Buffer or ddH_2O is added, stand the FATG Column for 3 min before centrifugation.

• **Column is clogged**
  ☐ Lysate contains insoluble residues
  → Remove insoluble residues by centrifugation.
  ☐ Sample is too viscous
  → Reduce the sample volume.
  ☐ Insufficient activity of Proteinase K
  → Use a fresh or well-stored Proteinase K stock solution and do not add Proteinase K into FATG2 Buffer directly.

• **Poor quality of genomic DNA -A_{260}/A_{280} ratio of eluted DNA is low**
  ☐ Poor cell lysis because of insufficient Proteinase K activity
  → Use a fresh or well-stored Proteinase K stock solution
  → Do not add Proteinase K into FATG2 Buffer directly.
  ☐ Poor cell lysis because of insufficient mixing with FATG2 buffer
  → Mix the sample and FATG2 Buffer immediately and thoroughly by pulse-vortexing.
  ☐ Poor cell lysis because of insufficient incubation time
  → Extend incubation time and make sure that no residual particle remain.
  ☐ Degradation of eluted DNA - Genomic DNA extracted from paraffin-embedded tissue or sample is old
  → Always use fresh or well-stored sample for genomic DNA extraction.