User Manual

FavorPrep™ FFPE Tissue DNA Extraction Micro Kit

-For extraction DNA from paraffin-fixed tissues

For Research Use Only

Kit Contents:

Cat. No:	FAFFM004B (4 preps)	FAFFM050B (50 preps)	FAFFM100B (100 preps)
FATG1 Buffer	1.5 ml	15 ml	30 ml
FATG2 Buffer	1.5 ml	15 ml	30 ml
Proteinase K (Liquid)	100 µl	1050 µl	1050 µl × 2
W1 Buffer * (Contentrate)	1.3 ml	22 ml	44 ml
Wash Buffer ** (Concentrate)	1 ml	10 ml	20 ml
Elution uffer	1 ml	15 ml	30 ml
TG Micro Column Δ	4 pcs	10 pcs × 5	10 pcs × 10
Collection Tube	4 pcs	50 pcs	100 pcs
Elution Tube	4 pcs	50 pcs	100 pcs
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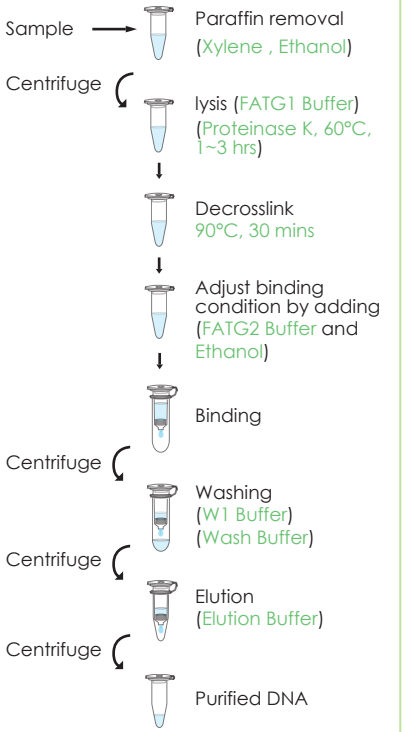
Δ Store the TG Micro Columns to 4~8°C upon receipt.

Preparation of W1 Buffer and Wash Buffer by adding ethanol (96~100%).			
* Ethanol volume for W1 Buffer	0.5 ml	8 ml	16 ml
**Ethanol volume for Wash Buffer	4 ml	40 ml	80 ml

Brief procedure:



Specification:

Principle: micro spin column (silica matrix)
Minimum elution volume: 12 µl
Sample size: ≤25 mg fixed tissue

Important Notes:

1. **Additional requirement :** Xylene, RNase A (optional), 96~100% ethanol.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. Add ethanol (96~100%) to **W1 Buffer** and **Wash Buffer** at the first open.
4. Prepare dry or water baths before the operation: one set up at 60°C for step 10; the other set up at 90°C for step 12.
5. Preheat the Elution Buffer to 65°C for step 19.
6. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.

Protocol: Isolation of DNA from paraffin-fixed tissue

Please Read Important Notes Before Starting Following Steps.

1. Add up to 25 mg of paraffin slice sample to a microcentrifuge tube.
2. Add 1 ml xylene and mix well. Close the lid and vortex vigorously for 10 secs. Incubate the sample at room temperature until the paraffin is dissolved completely.
3. Centrifuge at full speed for 5 mins. Remove the supernatant by pipetting.
4. Add 1 ml ethanol (96~100%) to the deparaffined tissue and mix gently by vortexing.
5. Centrifuge at full speed for 3 mins. Remove the supernatant by pipetting.
6. Repeat step 4 and 5.
7. Incubate at 37°C for 10~15 mins to evaporate ethanol residue completely.
8. Add 200 µl FATG1 Buffer and mix well.
9. Add 20 µl Proteinase K to the sample mixture. Mix thoroughly by vortexing.
10. **Incubate at 60°C until the tissue is lysed completely (1~3 hrs).** Vortex occasionally during incubation.
    -Sample can be incubated overnight as well for complete lysis.
11. **(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 mins.
12. **Incubate at 90°C for 30 mins.** Vortex occasionally during incubation.
13. Add 200 µl FATG2 Buffer to the sample mixture, mix thoroughly by pulse-vortexing.
14. Add 200 µl ethanol (96~100%) to the sample mixture. Mix thoroughly by pulse-vortexing.
15. Place a TG Micro Column in a Collection Tube. Transfer the mixture carefully to the TG Micro Column. Centrifuge at full speed (~18,000 x g) for 1 min **then place the TG Micro Column to a new Collection Tube.**
16. Add 400 µl W1 Buffer to the TG Micro Column. Centrifuge at full speed for 1 min then discard flow-through.
    -Make sure that ethanol has been added into W1 Buffer at the first open.
17. Add 650 µl Wash Buffer to the TG Micro Column. Centrifuge at full speed for 1 min then discard flow-through.
    -Make sure that ethanol has been added into Wash Buffer at the first open.
18. Centrifuge at full speed for an additional 3 mins to dry the column.
    -Important Step! This step will remove the residual liquid.
19. Add 12 µl of preheated Elution Buffer or ddH_2O (pH 7.5-9.0) to the membrane of the TG Micro Column. Stand the TG Micro Column for 3 mins.
    -Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
20. Centrifuge at full speed for 2 mins to elute DNA.