# fisher biotec au stralia

**Brief procedure:** 

Australian distributors: Fisher Biotec Australia free call: 1800 066 077 email: info@fisherbiotec.com web: www.fisherbiotec.com



## FavorPrep<sup>TM</sup> FFPE Tissue DNA Extraction Micro Kit

-- For extraction DNA from paraffin-fixed tissues

For Research Use Only

#### **Kit Contents:**

Cat. No:	FAFFM004 (4 preps)	FAFFM050 (50 preps)	FAFFM100 (100 preps)
FATG1 Buffer	1.5 ml	15 ml	30 ml
FATG2 Buffer	1.5 ml	15 ml	30 ml
Proteinase K (lyophilized)	1 mg	11 mg	11 mg x 2
W 1 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	4 pcs	50 pcs	100 pcs
Collection Tube	8 pcs	100 pcs	200 pcs
Elution Tube	4 pcs	50 pcs	100 pcs
User Manual	1	1	1
Preparation of Proteinase K solution (10 mg/ml) by adding ddH2O			
ddH2O volume for Proteinase K	0.1 ml	1.1 ml	
Preparation of W 1 Buffer and Wash Buffer by adding ethanol (96 ~ 100%)			
* Ethanol volume for W 1 Buffer	0.5 ml	8 ml	16 ml
**Ethanol volume for Wash Buffer	4 ml	40 ml	80 ml

paraffin removal Sample (Xylene, Ethanol) centrifuge lysis (FATG1 Buffer) (Proteinase K,60 °C, 1~3 hr) ţ Decrosslink 90 °C, 30 min 1 Adjust binding condition by adding (FATG2 Buffer and Ethanol) I Binding centrifuge ( Washing (W1 Buffer) (Wash Buffer) centrifuge Flution (Elution Buffer) centrifuge Pure DNA

#### Specification:

Principle: mini 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 1.1 ml sterile ddH2O 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.
- 4. Add ethanol (96-100%) to W1 Buffer and Wash Buffer when first open.
- 5. Prepare dry baths or water baths before the operation: one to 60 °C for step 10 and the other to 90 °C for step 12.
- 6. Preheat the Elution Buffer to 65 °C for step 19.
- 7. 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 sec. Incubate the sample at room temperature until the paraffin is dissolved completely.
- 3. Centrifuge at full speed for 5 min. 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 min. Remove the supernatant by pipetting.
- 6. Repeat step 4 and 5.
- 7. Incubate at 37 °C for 10 ~15 min to evaporate ethanol residue completely.
- 8. Add 200  $\mu l$  FATG1 Buffer and mix well.
- 9. Add 20 µl Proteinase K (10mg/ml) to the sample mixture. Mix thoroughly by vortexing.
- 10. 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.
- 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 min.
- 12. Incubate at 90 °C for 30 min. Vrotex 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 when 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 when first open.
- Centrifuge at full speed for an additional 3 min to dry the column.
  Important Step! This step will remove the residual liquid.
- Inportant step: This step will remove the restaudingula.
  Add 12 μl of preheated Elution Buffer or ddH2O (pH 7.5-9.0) to the membrane of the TG Micro Column. Stand the TG Micro
  - 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.
- 20. Centrifuge at full speed for 2 min to elute DNA.