User Manual

FavorPrep™ Tissue Genomic DNA Extraction Mini Kit

-For extraction genomic DNA from animal cells, animal tissues, blood, bacteria, paraffin fixed tissue, yeast and fungi

For Research Use Only

Kit Contents:

Cat. No:	FATGK 000 (4 preps)	FATGK 001 (50 preps)	FATGK 001-1 (100 preps)	FATGK 001-2 (300 preps)
FATG1 Buffer	1.5 ml	15 ml	30 ml	70 ml
FATG2 Buffer	1.5 ml	15 ml	30 ml	70 ml
Proteinase K (Liquid)	100 µl	1050 µl	1050 µl × 2	1600 µl × 4
W1 Buffer * (Concentrate)	1.3 ml	22 ml	44 ml	124 ml
Wash Buffer ** (Concentrate)	1 ml	10 ml	20 ml	55 ml
Elution Buffer	1 ml	15 ml	30 ml	90 ml
FATG Mini Column	4 pcs	50 pcs	100 pcs	300 pcs
Collection Tube	8 pcs	100 pcs	200 pcs	600 pcs
Elution Tube	4 pcs	50 pcs	100 pcs	300 pcs
Micropestle	4 pcs	50 pcs	100 pcs	300 pcs
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Preparation of W1 Buffer and Wash Buffer by adding ethanol (96~100%).				
* Ethanol volume for W1 Buffer	0.5 ml	8 ml	16 ml	45 ml
**Ethanol volume for Wash Buffer	4 ml	40 ml	80 ml	220 ml

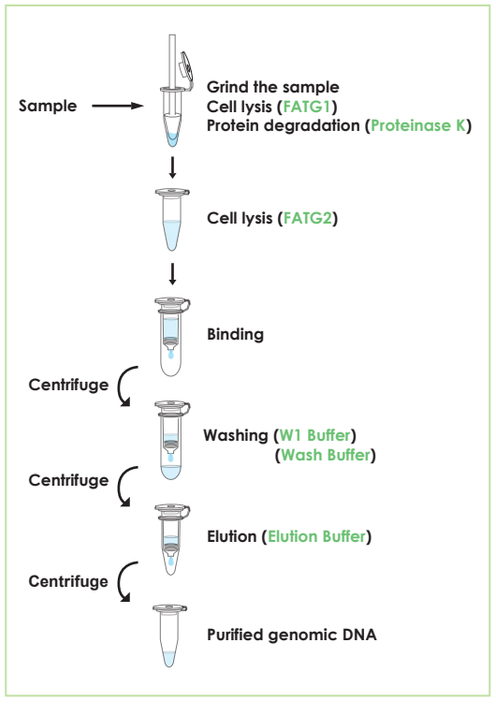
Specification:

Principle: mini spin column (silica matrix)
Operation time: 30~60 mins
Binding capacity: up to 60 µg DNA/column
Typical yield: 15~35 µg/prep
Column applicability: centrifugation and vacuum
Minimum elution volume: 50 µl
Sample size: <25 mg animal tissue;
1.2 cm mouse tail;
<10^7 cultured cells.

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Add ethanol (96~100%) to **W1 Buffer** and **Wash Buffer** at the first open.
3. Prepare dry or water baths before the operation.
4. Preheat the Elution Buffer to 70°C for elution step.
5. All centrifuge steps are perform at full speed (~18,000 x g) in a microcentrifuge.

Brief procedure:



Protocol: Isolation of DNA from Animal Tissue

Please Read Important Notes Before Starting Following Steps.

Additional requirement: RNase A (optional), 96~100% ethanol.

Hint: Set dry or water baths: 60°C for step 4 and 70°C for step 6.

1. Cut tissue sample (up to 25 mg) 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 to the sample mixture. Mix thoroughly by vortexing.
4. **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.
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 mins.
6. Add 200 µl FATG2 Buffer to the sample mixture, mix thoroughly by pulse-vortexing and **incubate at 70°C for 10 mins.**
7. Add 200 µl ethanol (96~100%) to the sample mixture. Mix thoroughly by pulse-vortexing.
8. Briefly spin the tube to remove drops which 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 at the 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 at the first open.
12. Centrifuge at full speed for an additional 3 mins 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 mins.
    -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 mins to elute DNA.

Protocol: Isolation of DNA from Animal Cultured Cells

Please Read Important Notes Before Starting Following Steps.

Additional requirement: RNase A (optional), 96~100% ethanol, trypsin or cell scraper (for monolayer cell), PBS.

Hint: Set dry or water baths: 60°C and 70°C.

1. Harvest cells
   a. Cells grown in suspension
      i. Transfer the appropriate number of cells (up to 1×10^7) to a microcentrifuge tube.
      ii. Centrifuge at 300 x g for 5 mins. Discard supernatant carefully and completely.
   b. Cells grown in monolayer
      i. Detach cells from the dish or flask by trypsinization or using a cell scraper. Transfer the appropriate number of cell (up to 1×10^7) to a microcentrifuge tube.
      ii. Centrifuge at 300 x g for 5 mins. Discard supernatant carefully and completely.
2. Resuspend cell pellet in PBS to a final volume of 200 µl.
3. Follow the Animal Tissue Protocol starting from step 2.

Protocol: Isolation of Genomic DNA and Viral DNA from Blood

Please Read Important Notes Before Starting Following Steps.

Additional requirement: RNase A (optional), 96~100% ethanol, PBS.

Hint: Set dry or water baths: 60°C for step 3 and 70°C for step 4.

1. Transfer up to 200 µl sample (whole blood, serum, plasma, body fluids, buffy coat) to a microcentrifuge tube.
   -If the sample volume is less than 200 µl, add the appropriate volume of PBS.
2. **(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.
3. Add 20 µl Proteinase K to the sample, and then add 200 µl FATG2 Buffer to the sample. Mix thoroughly by pulse-vortexing. Incubate at 60°C for 30 mins. Vortex occasionally during incubation.
4. Incubate at 70°C for 10 mins.
5. Follow the Animal Tissue Protocol starting from step 7.

Protocol: Isolation DNA from Bacteria

Please Read Important Notes Before Starting Following Steps

Additional requirement: • RNase A (optional), 96~100% ethanol.
• For Gram-positive bacteria: lysozyme reaction solution (20 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton) is suggested.

Hint: Set dry or water baths: one to 60°C, the other to 70°C.

I. For bacterial cultures

1. Transfer 1 ml well-grown bacterial culture to a microcentrifuge tube (not provided).
2. Descend the cells by centrifuging at full speed for 2 mins and discard supernatant completely.
3. Follow the Animal Tissue Protocol starting from step 2.

II. For bacterial in biological fluids

1. Collect cells by centrifuging biological fluids at 7,500 rpm (5,000 x g) for 10 mins and discard supernatant completely.
2. Follow the Animal Tissue Protocol starting from step 2.

III. For bacteria from eye, nasal, pharyngeal, or other swabs

1. Soak the swabs in 2 ml PBS at room temperature for 2~3 hrs.
2. Collect cells by centrifuging at 7,500 rpm (5,000 x g) for 10 mins and discard supernatant completely.
3. Follow the Animal Tissue Protocol starting from step 2.

IV. For Gram-positive bacteria

Hint: Set dry or water baths: one to 37°C, another to 60°C and the other to 95°C.

1. Transfer 1 ml well-grown bacterial culture to a microcentrifuge tube (not provided).
2. Descend the cells by centrifuging at full speed for 2 mins and discard supernatant completely.
3. Resuspend the cell pellet in **200 µl lysozyme reaction solution** (20 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton). Incubate at 37°C for 30~60 mins.
4. **(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.
5. Add 20 µl Proteinase K to the sample, and then add 200 µl FATG2 Buffer to the sample. Mix thoroughly by pulse-vortexing. Incubate at 60°C for 30 mins and vortex occasionally during incubation.
6. Do a furter incubation at 95°C for 15 mins.
7. Follow the Animal Tissue Protocol starting from step 7.

Protocol: Isolation of DNA from Yeast

Please Read Important Notes Before Starting Following Steps.

Additional requirement: • RNase A (optional), 96~100% ethanol.
• Zymolyase or lyticase, 200 U for one preparation.
• Sorbitol buffer (1 M sorbitol; 100 mM EDTA; 14 mM ß-mercaptoethanol).

Hint: Set dry or water baths: one to 30°C, another to 60°C and the other to 70°C.

1. Transfer 3 ml log-phase (OD600=1) yeast culture to a microcentrifuge tube (not provided).
2. Descend the cells by centrifuging at 7,500 rpm (5,000 x g) for 10 mins. Discard supernatant completely.
3. Resuspend the cell pellet in 600 µl **sorbitol buffer** (1 M sorbitol; 100 mM EDTA; 14 mM ß-mercaptoethanol). Add 200 U zymolyase or lyticase and incubate at 30°C for 30 mins.
4. Centrifuge at 7,500 rpm (5,000 x g) for 5 mins. Remove supernatant.
5. Follow the Animal Tissue Protocol starting from step 2.

Protocol: Isolation of DNA from Dried Blood Spot

Please Read Important Notes Before Starting Following Steps.

Additional requirement: • RNase A (optional), 96~100% ethanol.

Hint: Set dry or water baths: one to 85°C, another to 60°C and the other to 70°C.

1. Cut the filter paper (e.g. S&S 903) with dried blood spot into a microcentrifuge tube. Add 200 µl FATG1 Buffer and incubate at 85°C for 10 mins.
2. Add 20 µl Proteinase K to the sample mixture. Mix thoroughly by vortexing. Incubate at 60°C for 1 hr. Vortex occasionally during incubation.
3. Follow the Animal Tissue Protocol starting from step 6.

Protocol: Isolation of DNA from Fixed Tissue

Please Read Important Notes Before Starting Following Steps.

Additional equipment: • RNase A (optional), 96~100% ethanol.
• Xylene.

Hint: Set dry or water baths: one to 37°C, another to 60°C and the other to 70°C.

I. For paraffin-embedded tissues

1. Cut up to 25 mg paraffin-embedded tissue sample to a microcentrifuge tube (not provided).
2. Add 1 ml xylene, mix well and incubate at room temperature for 30 mins.
3. Centrifuge at full speed for 5 mins. Remove supernatant.
4. Add 1 ml ethanol (96~100%) to the deparaffined tissue, mix gently by vortexing.
5. Centrifuge at full speed for 3 mins. Remove supernatant by pipetting.
6. Repeat step 4 and 5.
7. Incubate at 37°C for 10~15 mins to evaporate ethanol residue completely.
8. Grind the tissue sample by micropestle or liquid nitrogen and follow the Animal Tissue Protocol starting from step 2.

II. For formalin-fixed tissues

1. Wash 25 mg tissue sample twice with 1 ml PBS to remove formalin.
2. Grind the tissue sample by micropestle or liquid nitrogen and follow the Animal Tissue Protocol starting from step 2.

Troubleshooting

Problem/Possible reasons	Solutions
• Low or no yield of genomic DNA	
Low amount of cells in the sample	Increase the sample size or concentrate a larger sample volume to 200 µl.
Too much amount of sample was used	Reduce the sample volume.
Poor cell lysis	
Poor cell lysis because of insufficient Proteinase K activity	Do not add Proteinase K into FATG2 Buffer directly.
	Make sure the reactive temperature and time is correct.
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 remains.
Insufficient binding of DNA to column's membrane	
Ethanol is not added into sample lysate before DNA binding	Make sure that the correct volume 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 Wash Buffer	
The percentage of ethanol is not correct in Wash Buffer	Make sure that the correct volume of ethanol (96~100%) is added into Wash Buffer at the 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 5 mins before centrifugation.
• Column is clogged	
Lysate contains insoluble residues	Remove insoluble residues (e.g., bone or hair) by centrifugation.
Sample is too viscous	Reduce the sample volume.
Insufficient activity of Proteinase K	Make sure the reactive temperature and time is correct; do not add Proteinase K into FATG2 Buffer directly.
• Poor quality of genomic DNA	
A260/A280 ratio of eluted DNA is low	
Poor cell lysis because of insufficient Proteinase K activity	Make sure the reactive temperature and time is correct.
	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 the incubation time and make sure that no residual particulates remain.
A260/A280 ratio of eluted DNA is high	
A lot of residual RNA in eluted DNA	Follow the Animal Tissue Protocol step 5 to remove RNA.
FATG2 Buffer was added into sample lysate before added RNase A	Make sure that RNase A has been added to the sample lysate before adding FATG2 Buffer when using optional RNase A step.
Degradation of eluted DNA	
Sample is old	Always use fresh or well-conserved sample for genomic DNA extraction.
	Genomic DNA extracted from paraffin-embedded tissue is usually degraded. It is still suitable for PCR reaction, but is not recommended for Southern blotting and restriction enzyme analysis.
Buffer for gel electrophoresis contaminated with DNase	Use fresh running buffer for gel electrophoresis.