User Manual

FavorPrep™ 96-well Genomic DNA Kit

For Research Use Only

-For 96-well high-throughput DNA extraction from whole blood, buffy coat, serum, plasma, body fluids, cultured cells and animal tissues.

Kit Contents:

Cat. No.: (Quantity)	FADWE 96001 (1 plate)	FADWE 96002 (2 plates)	FADWE 96004 (4 plates)
FATG1 Buffer	40 ml	80 ml	80 ml × 2
FATG2 Buffer	40 ml	80 ml	80 ml × 2
W1 Buffer * (Concentrate)	44 ml	88 ml	88 ml × 2
Wash Buffer ■ (Concentrate)	17.5 ml	35 ml	35 ml × 2
Elution Buffer	30 ml	60 ml	60 ml × 2
Proteinase K (Liquid)	1050 µl × 2	1050 µl × 4	1050 µl × 8
Filter Plate (96-Well DNA Binding Plate)	1 plate	2 plates	4 plates
Collection Plate (96-Well 2 ml Plate)	3 plate	6 plates	12 plates
Elution Plate (96-Well PCR Plate)	1 plate	2 plates	4 plates
Adhesive Film	2 pcs	4 pcs	8 pcs
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Preparation of working buffers

Add RNase-free ethanol (96~100%) to Wash Buffer 1 and Wash Buffer 2 for the first use.

	FADWE 96001	FADWE 96002	FADWE 96004
* Ethanol volume for Wash Buffer 1	16 ml	32 ml	
■ Ethanol volume for Wash Buffer 2	70 ml	140 ml	

Quality Control

The quality of 96-Well Genomic DNA Kit is tested on a lot-to-lot basis. The purified DNA is checked by real-time PCR and capillary electrophoresis.

Specification:

Principle: 96- well DNA Binding Plate (silica membrane)
Sample size/preparation : up to 200 µl of fresh/frozen whole blood, buffy coat, serum, plasma, body fluids
up to 25 mg of animal tissue
up to 5×10^6 cultured animal cells
Processing: centrifugation protocol or vacuum & centrifugattion protocol
Operation time: <90 mins/96 preparations (1 plate)
DNA Binding capacity: up to 30 µg/well
Elution volume: 75~200 µl

Reagent to be provided by user

1. 96~100% ethanol
2. (Optional) RNAse A (50 mg/ml)

Important notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. The maximum sample size is described on specification, do not use the sample more than the limitation.
3. Add ethanol (96~100%) to W1 Buffer and Wash Buffer at the first open.
4. Set incubator or ovens to 60°C and 70°C before the operation.
5. Preheat the Elution Buffer to 70°C for DNA elution.
6. Equipments required:
   - 60°C and 70°C shaker incubators or ovens.
   - For centrifugation protocol: A centrifuge is required, capable of 5,600~6,000 x g, with a swing-bucket rotor and the adaptor for 96-well plates.
   - For vacuum protocol: A vacuun manifold for 96-well plate and a vacuum source reached to 15 inches Hg are required. ***(Alternative)***: If using centrifugation for Elution Step (STEP 6), a centrifuge equipment is required, capable of 5,600~ 6,000 x g, with a swing-bucket rotor and the adaptor for 96-well plate.

Sample amount and yield

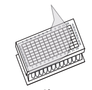
Sample (maximum sample size)	Recommended amount of sample used	Average yield (µg)
Whole Blood (up to 200 µl)	200 µl	4~12
Low yield Tissue (Mouse)(up to 25 mg)	Heart, 25 mg	5~15
	Brain, 25 mg	5~25
	Kidney, 25 mg	20~30
	Lung, 25 mg	5~10
	Intestine, 10 mg	5~10
High yield Tissue (Mouse)(up to 10 mg)	Spleen, 10 mg	5~30
Animal cells (up to 5×10^6 cells)	5×10^6 cells	15~20

Brief procedure:

● STEP 1. Sample preparation and lysis

For whole blood, buffy coat, serum, plasma, body fluids

• Add Proteinase K in each well of Collection Plate (first Collection Plate). → • Add sample. → • Add FATG2 Buffer and mix completely. → • Sealing with Adhesive Film. → • Incubate the plate with shaking at 60°C for 20 mins.



For aminam tissues and cultured cells

• Collect samples in a Collection Plate (first Collection Plate). → • Add FATG1 Buffer and Proteinase K. → • Seal with Adhesive Film. Incubate the plate with shaking at 60°C for 1~2 hrs. → • Add FATG2 Buffer and mix completely. → • Seal with Adhesive Film. Further incubate the plate at 70°C for 20 mins.

● STEP 2. Adjust binding condition:

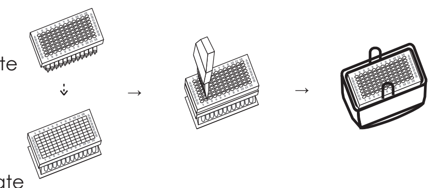
• Add ethanol. → • Mix by pipetting.

or

● STEP 3. Bind DNA to Filter Plate:

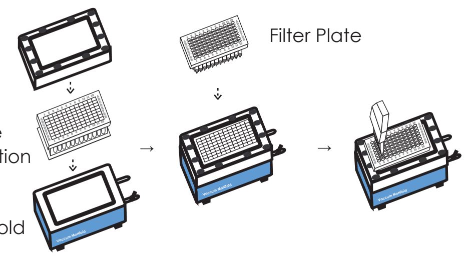
Centrifuge protocol

• Combind the plates.
• Transfer the sample mixture to Filter Plate.
• Centrifuge at 4,500~6,000 x g for 5 mins.



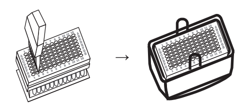
Vacuum protocol

• Assemble plates to vacuum manifold.
• Transfer the sample mixture to Filter Plate.
• Apply 10 inches Hg vacuum until the well have emptied.

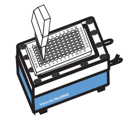


● STEP 4. Wash the Filter Plate Twice (W1 Buffer and Wash Buffer)

Centrifuge protocol:
• Add W1 Buffer. Centrifuge at 5,600~6,000 x g for 2 mins.
• Add Wash Buffer. Centrifuge at 5,600~6,000 x g for **15 mins.**



Vacuum protocol:
• Add W1 Buffer. Apply vacuum at 10 inches Hg.
• Add Wash Buffer. Apply vacuum at 10 inches Hg for **10 mins**.



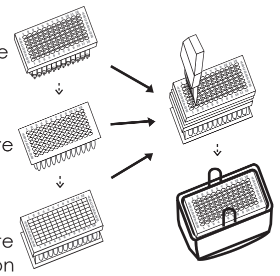
● STEP 5. Dry the membranes of Filter Plate:

Centrifuge protocol:
• Stand the Filter Plate on a clean paper towel at room temperature for 10 mins.

Vacuum protocol:
• Tap the Filter Plate tips on paper towel.
• Return the Filter Plate and the Collection Plate to the manifold.
• Apply maximum vacuum for an additional 10 mins.

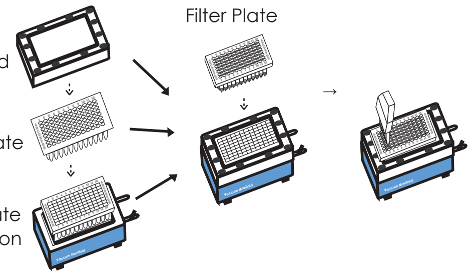
● STEP 6. DNA Elution:

Centrifuge protocol:
• Add Elution Buffer or ddH_2O to the Filter Plate. Stand for 3 mins.
• Centrifuge to elute DNA.



Vacuum protocol:
• Add Elution Buffer or ddH_2O to the Filter Plate. Stand for 3 mins.
• Close the manifold valve. Turn on the vacuum source to build up a vacuum to 15 inches Hg.
• Open the manifold valve to apply vacuum to elute DNA.

$\boxed{\text{Alternative}}$ **: If the consistent volume of elutes are needed, the centrifuge protocol are recommended to perform the elution step. (Page 3, STEP 6)**



Protocol: Centrifugation processing
Please Read Important Notes Before Starting The Following Steps.

Required hardware
- 60°C and 70°C shaker incubators or ovens.
- Centrifuge equipment capable of 5,600~6,000 x g with a swing-bucket rotor and the adaptor for 96-well plate.

Hint
Preheat required Elution Buffer or ddH_2O (75~100 µl per well) to 70°C for DNA elution step (STEP 6).

STEP 1: Sample lysis

•Whole blood, buffy coat, serum, plasma, body fluids
- Add 20 µl Proteinase K to each wells of a Collection Plate (provided, 96-well 2 ml Plate; first Collection Plate).
- Add 200 µl of sample to each well and mix by pipetting.
- Add 200 µl FATG2 Buffer to each well and mix by pipetting.
- Seal with Adhesive Film. Incubate the plate with shaking at 100 rpm, 60°C for 20 mins.
- **(Optional)** If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ml, not provided) to each well and incubate at room temperature for 4 mins.
- Proceed to STEP 2.

•Animal Tissue
- Cut up to 25 mg of animal tissues (or 0.5 cm of mouse tail) and transfer into each well of a Collection Plate (provided, 96-well 2 ml Plate; first Collection Plate). Reduce the sample size to 10 mg for high DNA contained tissue, such as liver and spleen.
- Add 200 µl FATG1 Buffer and 20 µl Proteinase K to each well of a Collection Plate (provided, 96-well 2 ml Plate; first Collection Plate).
- Seal with Adhesive Film. Incubate the plate with shaking at 100 rpm, 60°C for 1~2 hrs or more time until the tissue sample is lysed completely. To reduce the incubation time, grind the sample in liquid nitrogen or homogenize the sample mechanically in advance.
- **(Optional)** If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ml, not provided) to each well and incubate at room temperature for 4 mins.
- Add 200 µl FATG2 Buffer to each well and mix by pipetting.
- Seal with Adhesive Film. Incubate the plate with shaking at 100 rpm, 70°C for 20 mins until the sample lysate is clear.
- Proceed to STEP 2.

•Animal Cultured Cell
- Transfer cultured cells to each well of a Collection Plate (provided, 96-well 2 ml Plate; first Collection Plate).
- Centrifuge at 1,000 x g for 10 mins to pellet the cells, discard the supernatant.
- Add 200 µl FATG1 Buffer and 20 µl Proteinase K to each well and resuspend the pellet by pipetting.
- Seal with adhesive film and incubate the plate with shaking at 100 rpm, 60°C for 10~20 mins to lyse the sample.
- **(Optional)** If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ml, not provided) to each well and incubate at room temperature for 4 mins.
- Add 200 µl FATG2 Buffer to each well and mix by pipetting.
- Seal with Adhesive Film. Incubate the plate with shaking at 100 rpm, 70°C for 20 mins until the sample lysate is clear.
- Proceed to STEP 2.

STEP 2. Adjust binding condition:
- Add 200 µl ethanol (96~100%) to each well. Mix immediately by pipetting 5~10 times.

STEP 3. DNA Binding
- Place a Filter Plate (provided, 96-Well DNA Binding Plate) on a clean Collection Plate (provided, second Collection Plate).
- Transfer the sample mixture to each well of the Filter Plate and discard the Collection Plate (first Collection Plate).
- Place the plates in a rotor bucket and centrifuge at 5,600~6,000 x g for 2 mins.
- Discard the flow-through and return the Filter Plate to the Collection Plate.

STEP 4. Wash the Filter Plate with W1 Buffer and Wash Buffer
- Add 400 µl of W1 Buffer (ethanol added) to each well of the Filter Plate.
- Place the combined plate in a rotor bucket and centrifuge at 5,600~6,000 x g for 2 mins.
- Discard the flow-through and return the Filter Plate to the Collection Plate.
- Add 650 µl of Wash Buffer (ethanol added) to each well of the Filter Plate.
- Place the combined plate in a rotor bucket and centrifuge at 5,600~6,000 x g for 15 mins.
- Discard the flow-through and return the Filter Plate to the Collection Plate.

STEP 5. Dry the membranes of Filter Plate
- Place the Filter Plate on top of a clean paper towel (not provided) and stand at room temperature for 10 mins.

STEP 6. DNA Elution
- Place an Elution Plate (provided, 96-Well PCR Plate) on top of a clean Collection Plate (provided, third Collection Plate) then place the Filter Plate on the Elution Plate. (top: Filter Plate; middle: 96-well PCR Plate; bottom: Collection Plate).
- Add 75~200 µl of preheated Elution Buffer or ddH_2O to the membrane center of the Filter Plate. Stand for 3 mins.
  -Important Step! For effective elution, make sure that Elution Buffer or ddH_2O is dispensed on the membrane center and is absorbed completely.
  -Important: Do not elute the DNA using Elution Buffer or ddH_2O less than suggested volume (<75 µl). It will lower the DNA yield.
- Place the plates in a rotor bucket and centrifuge at 5,600~6,000 x g for 5 mins to elute DNA.
- Seal the Adhesive Film and store the DNA at -20°C.

Protocol: Vacuum processing
Please Read Important Notes Before Starting The Following Steps.

Required hardware
- 60°C and 70°C shaker incubators or ovens.
- Vacuun manifold for 96-well plate and vaccum source reached to -15 inches Hg.
  Alternative: If using centrifugation for Elution Step (STEP 6), a centrifuge equipment is required, capable of 5,600~6,000 x g, with a swing-bucket rotor and the adaptor for 96-well plate.

Hint
Preheat required Elution Buffer or ddH_2O (75~100 µl per well) to 70°C for DNA elution step (STEP 6).

STEP 1: Sample lysis

•Whole blood, buffy coat, serum, plasma, body fluids
- Add 20 µl Proteinase K to each well of a Collection Plate (provided, 96-well 2 ml Plate; first Collection Plate).
- Add 200 µl of sample to each well and mix by pipetting.
- Add 200 µl FATG2 Buffer to each well and mix by pipetting.
- Seal with Adhesive Film. Incubate the plate with shaking at 100 rpm, 60°C for 20 mins.
- **(Optional)** If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ml, not provided) to each well and incubate at room temperature for 4 mins.
- Proceed to STEP 2.

•Animal Tissue
- Cut up to 25 mg of animal tissues (or 0.5 cm of mouse tail) and transfer into each well of a Collection Plate (provided, 96-well 2 ml Plate; first Collection Plate). Reduce the sample size to 10 mg for high DNA contained tissue, such as liver and spleen.
- Add 200 µl FATG1 Buffer and 20 µl Proteinase K to each well of a Collection Plate (provided, 96-well 2 ml Plate; first Collection Plate).
- Seal with Adhesive Film. Incubate the plate with shaking at 100 rpm, 60°C for 1~2 hrs or more time until the tissue sample is lysed completely. To reduce the incubation time, grind the sample in liquid nitrogen or homogenize the sample mechanically in advance.
- **(Optional)** If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ml, not provided) to each well and incubate at room temperature for 4 mins.
- Add 200 µl FATG2 Buffer to each well and mix by pipetting.
- Seal with Adhesive Film. Incubate the plate with shaking at 100 rpm, 70°C for 20 mins until the sample lysate is clear.
- Proceed to STEP 2.

•Animal Cultured Cell
- Transfer cultured cells to each well of a Collection Plate (provided, 96-well 2 ml Plate; first Collection Plate).
- Centrifuge at 1,000 x g for 10 mins to pellet the cells, discard the supernatant.
- Add 200 µl FATG1 Buffer and 20 µl Proteinase K to each well and resuspend the pellet by pipetting.
- Seal with adhesive film and incubate the plate with shaking at 100 rpm, 60°C for 10~20 mins to lyse the sample.
- **(Optional)** If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ml, not provided) to each well and incubate at room temperature for 4 mins.
- Add 200 µl FATG2 Buffer to each well and mix by pipetting.
- Seal with Adhesive Film. Incubate the plate with shaking at 100 rpm, 70°C for 20 mins until the sample lysate is clear.
- Proceed to STEP 2.

STEP 2: Adjust binding condition:
- Add 200 µl ethanol (96~100%) to each well. Mix immediately by pipetting 5~10 times.

STEP 3. DNA Binding
- Fix a clean Collection Plate (provided, second collection plate) on the rack of vacuum manifold and cover the manifold lid. Place a Filter Plate (provided, 96-Well DNA Binding Plate) on top of the Collection Plate.
- Transfer the sample mixture to the Filter Plate and discard the Collection Plate (first Collection Plate).
- Apply vacuum at 10 inches Hg until the well have emptied.
- Discard the flow-through and return the Filter Plate and the Collection Plate to the manifold.

STEP 4. Wash the Filter Plate with W1 Buffer and Wash Buffer
- Add 400 µl of Wash Buffer 1 (ethanol added) to each well of the Filter Plate.
- Apply vacuum at 10 inches Hg until the well have emptied.
- Discard the flow-through and return the Filter Plate and the Collection Plate to the manifold.
- Add 650 µl of Wash Buffer 2 (ethanol added) to each well of the Filter Plate.
- Apply vacuum at 10 inches Hg until the well have emptied.
- Discard the flow-through and return the Filter Plate and the Collection Plate to the manifold.

STEP 5. Dry the membranes of Filter Plate
- Gently tap the tips of the Filter Plate on a clean paper towel to remove residual liquid.
- Return the Filter Plate to the Collection Plate fixed in the manifold.
- Apply vacuum for an addition 10 mins.
- Discard the flow-through and the Collection Plate (third Collection Plate).

STEP 6. DNA Elution
- Place an Elution Plate (provided, 96-Well PCR Plate) on top of a clean Collection Plate (provided, third Collection Plate) and fix plates on the rack of manifold. Cover the manifold lid and place the Filter Plate on the Elution Plate. (top: Filter Plate; middle: 96-well PCR Plate; bottom: Collection Plate)
- Add 75~200 µl of preheated Elution Buffer or ddH_2O to the membrane center of the Filter Plate. Stand for 3 mins.
  -Important Step! For effective elution, make sure that Elution Buffer or ddH_2O is dispensed on the membrane center and is absorbed completely.
  -Important: Do not elute the DNA using Elution Buffer or ddH_2O less than suggested volume (<75 µl). It will lower the DNA yield.
- Close the manifold valve. Turn on the vacuum source to build up a vacuum to 15 inches Hg.
- Open the manifold valve to apply vacuum to elute DNA.
- Seal the Adhesive Film and store the RNA at -20°C.

Alternative: If the consistent volume of elutes are needed, the centrifuge protocol are recommended to perform the elution step (Page 3, STEP 6).