FavorPrep™ 96-well Genomic DNA Kit

(For Research Use Only)

Kit Contents/ Cat. No.:

	FADWE96001 1 plate	FADWE96002 2 plates	FADWE96004 4 plates
FATG1 Buffer	30 ml	65 ml	65 ml X 2
FATG2 Buffer	30 ml	65 ml	65 ml X 2
W1 Buffer	33 ml $^{\Phi}$	66 ml $^{\Phi\Phi}$	66 ml X 2 $^{\Phi\Phi}$
Wash Buffer (concentrated)	15 ml*	35 ml**	35 ml X 2 **
Elution Buffer	30 ml	60 ml	60 ml X 2
Proteinase K	24 mg†	48 mg††	48 mg X 2††
Filter Plate (96-Well DNA binding plate)	1 plate	2 plates	4 plates
96-Well 2 ml Plate	3 plate	6 plates	12 plates
96-Well PCR plate	1 plate	2 plates	4 plates
Adhesive Film	2 pcs	4 pcs	8 pcs

Φ,* Add required ethanol (96~100%) to W1 Buffer and Wash Buffer when first open.

	FADWE96001	FADWE96002 & FADWE96004
Ethanol volume for W1 Buffer	Φ 12 ml	$\Phi\Phi$ 24 ml / each bottle
Ethanol volume for Wash Buffer	* 60 ml	** 140 ml/ each bottle

† Add required ddH2O to Proteinase K bottle and disslove well. Store the prepared proteinase K at 4 °C.

	FADWE96001	FADWE96002 & FADWE96004
ddH2O volume for Proteinase K	† 2.4 ml	†† 4.8 ml/ each bottle

Quality Control

The quality of 96-Well Genomic DNA Kit is tested on a lot-to-lot basis. The purified DNA is checked by agarose gel analysis and quantified with spectrophotometer.

Specification:

Principle: 96- well DNA Binding Plate (silica membrane)
Sample size/ preparation : up to 200 µl of fresh/ frozen blood per well
up to 25 mg of animal tissue
up to 5 X 10^7 animal cultured cells
up to 10 bacterial cultured cells
Processing: centrifugation protocol or vacuum & centrifugattion protocol
Operation time: < within 90 min/ 96 preparation
DNA Binding capacity: up to 30 µg/ well
Elution volume: 100 ~200 µl

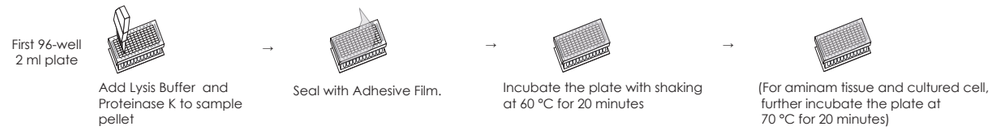
Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. The maxium 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 when first open.
4. Add ddH2O to proteinase K to prepare the 10 mg/ml proteinase K solution and store the solution at 4 °C
5. Prepare two dry baths or two water baths to 60 °C and 70 °C before the operation.
6. Preheat the Elution Buffer to 65 °C for DNA elution.

Brief procedure:

Material to be provided by user for a 96-well preparation
1. Centrifuge equiment with a swing -bucket rotor and adaptor for 96-well plate, capable of at least 5,600 ~ 6,000 X g.
2. Vacuun manifold for 96-well plate and a vaccum source
3. 96 ~100 % ethanol
4. 65 °C and 70 °C waterbaths or dry baths

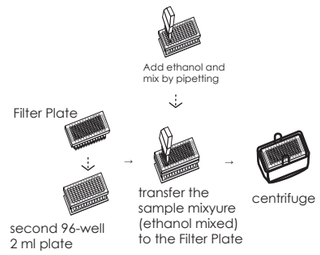
STEP 1: Sample Lysis



STEP 2: Bind DNA to Filter Plate:

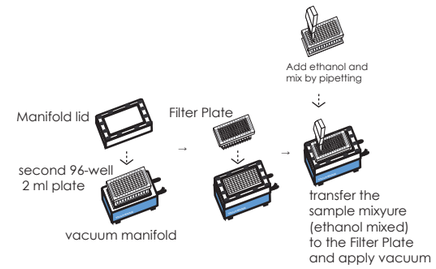
centrifuge protocol

· Add ethanol (96~100%) to each well of sample lysate. Mix immediately by pipetting 5~10 times.
· Assembly plates
· Transfer the sample mixture (ethanol mixed) to the Filter Plate.
· Place the combined plates in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.



vacuum protocol

· Add ethanol (96~100%) to each well of sample lysate. Mix immediately by pipetting 5~10 times.
· Place the plates on the rack of vacuum manifold.
· Transfer the sample mixture (ethanol mixed) to the Filter Plate.
· Apply vacuum until the wells have emptied.



STEP 3: Wash Filter Plate Twice: (W1 Buffer and Wash Buffer)

· Add W1 Buffer (ethanol added) to each well of the Filter Plate.
· Place the Filter plate combined with the second 96-well plate in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 15 min.
· Repeat STEP 3 with Wash Buffer to wash Filter Plate.

· Add W1 Buffer (ethanol added) to each well of the Filter Plate.
· Apply vacuum until the wells have emptied.
· Repeat STEP 3 with Wash Buffer to wash Filter Plate.

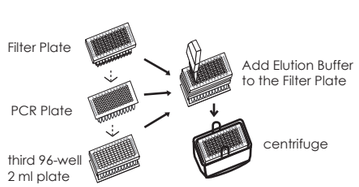
STEP 4: Dry the membranes of Filter Plate:

· Place the combined plate at 70 °C for 10 min.

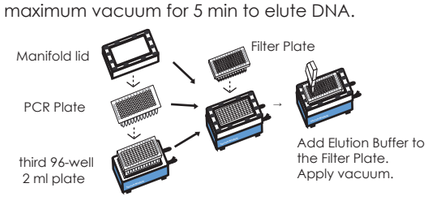
· Apply maximum vacuum for an additional 10 min.

STEP 5: DNA Elution:

· Assembly plates.
· Add Elution Buffer or ddH2O to the Filter Plate. Stand for 3 min.
· Centrifuge at 4,500 – 6,000 x g for 5 min to elute DNA.



· Gently tap the tips of the Filter Plate on paper towel to remove residual Wash Buffer.
· Assembly and place the plates on the rack of vacuum manifold .
· Add Elution Buffer or ddH2O to the Filter Plate. Stand for 3 min.
· Apply maximum vacuum for 5 min to elute DNA.



Protocol: (centrifugation processing)
- using centrifuging force to handle DNA binding step and washing steps.

Material to be provided by user for a 96-well preparation

1. Centrifuge equiment with a swing -bucket rotor and adaptor for 96-well plate, capable of at least 5,600 ~ 6,000 X g.
2. 96 ~100 % ethanol
3. Preheat required Elution Buffer (50~100 µl per well) at 60 °C. (For Step 4 DNA elution)

Please Read Important Notes Before Starting The Following Steps.

STEP 1: Sample lysis

- **Whole Blood**
  - Add 200 µl FATG2 Buffer and 20 µl Proteinase K (10 mg/ ml) to each well of the first 96-well 2 ml plate (provided).
  - Apply 200 µl of blood sample to each well and mix by pipetting. Seal with Adhesive Film.
  - Incubate at 60 °C for 20 min.
  - Proceed to STEP 2.
- **Animal Tissue**
  - Add 200 µl FATG1 Buffer and 20 µl Proteinase K (10 mg/ ml) to each well of the first 96-well 2 ml plate(provided).
  - Cut up to 25 mg of animal tissues (or 0.5 cm of mouse tail) and transfer into each well. Seal with Adhesive Film.
  - Incubate the plate with shaking at 60°C for 1~2 hours or more time until the tissue sample is lysed completely.
  - 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 min.
  - Add 200 µl FATG2 Buffer to each well and mix by shaking. Seal with Adhesive Film.
  - Incubate the plate with shaking at 70 °C for 20 min until the sample lysate is clear.
  - If there are insoluble material present following incubation, centrifuge the plate for 5 min at full speed and transfer the supernatants to a new 96-Well 2 ml plate (not provided).
  - Proceed to STEP 2.
- **Aminal Cultured Cell**
  - Transfer the cultured cells to each well of the first 96-well 2 ml plate (provided).
  - Centrifuge at 1,000 x g for 10 min to pellet the cells, discard the supernatant.
  - Add 200 µl FATG1 Buffer and 20 µl Proteinase K (10 mg/ ml) to each well and resuspend the pellet by pipetting.
  - Seal with adhesive film and incubate the plate with shaking at 60°C for 10~20 min to lyse the sample.
  - 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 min.
  - Add 200 µl FATG2 Buffer to each well. Seal with Adhesive Film and mix by shaking.
  - Incubate the plate with shaking at 70 °C for 20 min until the sample lysate is clear.
  - Proceed to STEP 2.

STEP 2: DNA Binding

- Add 200 µl ethanol (96~100%) to each well of sample lysate. Mix immediately by pipetting 5~10 times.
- Place a Filter Plate (96-Well Plasmid Plate) on top of the second 96-Well 2 ml Plate (provided).
- Remove the Adhesive Film from the first 96-well 2 ml plate. Transfer the clear lysate (supernatant) to the Filter Plate.
- Place the combined plate (Filter Plate combined with the second 96-Well 2 ml plate) in a rotor bucket and centrifuge at 4,500 – 6,000 x g for 5 min.
- Discard the flow-through and return the Filter Plate to the second 96-Well 2 ml Plate.

STEP 3: Wash Filter Plate Twice (W1 Buffer and Wash Buffer)

- Add 350 µl of W1 Buffer (ethanol added) to each well of the Filter Plate.
- Place the combined plate (Filter Plate combined with the second 96-Well 2 ml plate) in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 15 min.
- Add 650 µl of Wash Buffer (ethanol added) to each well of the Filter Plate.
- Place the combined plate (Filter Plate combined with the second 96-Well 2 ml plate) in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 15 min.
- Discard the second 96-Well 2 ml plate and the flow-through.

STEP 4: Dry the membranes of Filter Plate

- Place the Filter Plate on top of the third 96-Well 2 ml plate (provided) and incubate at 65 °C for 10 min.

STEP 5: DNA Elution

- Apart the combined plate of step 6.
- Place a 96-well PCR Plate (provided) on top of the third 96-Well 2 ml Plate and place the Filter Plate on the 96-Well PCR plate. (top: Filter Plate, middle: 96-well PCR Plate, bottom: 96-Well 2 ml plate)
- Add 100 ~ 200 µl of Elution Buffer or ddH_2O (pH8.0-8.5) to the membrane center of the Filter Plate. Stand for 3 min until Elution Buffer or ddH_2O has been absorbed by the membrane completely.
- Place the combined plate in a rotor bucket and centrifuge for 5 min at 4,500 – 6,000 x g for 5 min to elute DNA to the 96-well PCR plate.

Protocol: (Vacuum processing)
- using vacuum force to handle DNA binding step and washing steps.

Material to be provided by user for a 96-well preparation

1. Centrifuge equiment with a swing -bucket rotor and adaptor for 96-well plate, capable of at least 5,600 ~ 6,000 X g.
2. Vacuun manifold for 96-well plate and a vaccum source.
3. 96 ~100 % ethanol

Please Read Important Notes Before Starting The Following Steps.

STEP 1: Sample lysis

- **Whole Blood**
  - Add 200 µl FATG2 Buffer and 20 µl Proteinase K (10 mg/ ml) to each well of the first 96-well 2 ml plate (provided).
  - Apply 200 µl of blood sample to each well and mix by pipetting. Seal with Adhesive Film.
  - Incubate at 60 °C for 20 min.
  - Proceed to STEP 2.
- **Animal Tissue**
  - Add 200 µl FATG1 Buffer and 20 µl Proteinase K (10 mg/ ml) to each well of the first 96-well 2 ml plate(provided).
  - Cut up to 25 mg of animal tissues (or 0.5 cm of mouse tail) and transfer into each well. Seal with Adhesive Film.
  - Incubate the plate with shaking at 60°C for 1~2 hours or more time until the tissue sample is lysed completely.
  - 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 min.
  - Add 200 µl FATG2 Buffer to each well and mix by shaking. Seal with Adhesive Film.
  - Incubate the plate with shaking at 70 °C for 20 min until the sample lysate is clear.
  - If there are insoluble material present following incubation, centrifuge the plate for 5 min at full speed and transfer the supernatants to a new 96-Well 2 ml plate (not provided).
  - Proceed to STEP 2.
- **Aminal Cultured Cell**
  - Transfer the cultured cells to each well of the first 96-well 2 ml plate (provided).
  - Centrifuge at 1,000 x g for 10 min to pellet the cells, discard the supernatant.
  - Add 200 µl FATG1 Buffer and 20 µl Proteinase K (10 mg/ ml) to each well and resuspend the pellet by pipetting.
  - Seal with adhesive film and incubate the plate with shaking at 60°C for 10~20 min to lyse the sample.
  - 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 min.
  - Add 200 µl FATG2 Buffer to each well. Seal with Adhesive Film and mix by shaking.
  - Incubate the plate with shaking at 70 °C for 20 min until the sample lysate is clear.
  - Proceed to STEP 2.

STEP 2: DNA Binding

- Add 200 µl ethanol (96~100%) to each well of sample lysate. Mix immediately by pipetting 5~10 times.
- Place the scond 96-Well 2 ml plate (provided) on the rack of vacuum manifold and cover the manifold lid. And place a Filter Plate (96-well plasmid Plate, provided) on top of the second 96-Well 2 ml plate.
- Remove the Adhesive Film from the first 96-well 2 ml plate. Transfer the clear lysate (supernatant) to the Filter Plate.
- Apply vacuum until the wells have emptied.

STEP 3: Wash Filter Plate Twice (W1 Buffer and Wash Buffer)

- Add 350 µl of W1 Buffer (ethanol added) to each well of the Filter Plate.
- Apply vacuum until the wells have emptied.
- Add 650 µl of Wash Buffer (ethanol added) to each well of the Filter Plate.
- Apply vacuum until the wells have emptied.

STEP 4: Dry the membranes of Filter Plate

- Apply maximum vacuum for an additional 10 min to dry the membranes of Filter Plate.
- Discard the second 96-Well 2 ml plate and the flow-through.

STEP 5: DNA Elution

- Gently tap the tips of the Filter Plate on paper towel to remove residual Wash Buffer.
- place a 96-well PCR Plate (provided) on top of the third 96-Well 2 ml Plate (provided). Place the combined plate on the rack of vacuum manifold and cover the manifold lid.
- place the Filter Plate on top of the 96-well PCR Plate.
- Add 100 ~ 200 µl of Elution Buffer or ddH_2O (pH8.0-8.5) to the membrane center of the Filter Plate. Stand for 3 min until Elution Buffer or ddH_2O has been absorbed by the membrane completely.
- Apply maximum vacuum for 5 min to elute to the 96-well PCR plate.