

FavorPrepTM
Genomic DNA Mini Kit
(Blood/Cultured Cell)

User Manual

Cat. No.: FABGK 100 (100 Preps)
FABGK 300 (300 Preps)

For Research Use Only

Kit Contents

Cat. No. / preps	FABGK 100 (100 Preps)	FABGK 300 (300 Preps)
RBC Lysis Buffer	135 ml	405 ml
FATG Buffer	30 ml	75 ml
FABG Buffer	40 ml	100 ml
W1 Buffer	45 ml	130 ml
Wash Buffer* (concentrated)	25 ml	50 ml
Elution Buffer	30 ml	75 ml
FABG Column	100 pcs	300 pcs
2 ml Collection Tube	200 pcs	600 pcs

*Add 100 ml / 200 ml of ethanol (96~100%) to Wash Buffer when first open.

Specification

Sample Size: Up to 300 µl of Whole blood
Up to 200 µl of frozen blood
Up to 200 µl of buffy coat
Up to 1×10^7 of Cultured animal cells
Up to 1×10^9 of Cultured bacterial cells
Up to 5×10^7 of Fungus cells

Average Yield: about 50 µg

Format: spin column

Handling time: within 60 minutes

Important Notes

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. For Cat. No. FABGK 100, add 100 ml of ethanol (96~100%) to Wash Buffer when first open. For Cat. No. FABGK300, add 200 ml of ethanol (96~100%) to Wash Buffer when first open.

v. Add 200 µl of FATG Buffer to the tube and resuspend the cell pellet by vortex or pipetting.

vi. Incubate at room temperature for 5 minutes, and then follow the Cultured Cell Protocol starting from Step 2 (Cell Lysis).

Troubleshooting:

Low yield

- Too many cells were used
--reduce the sample volume.
- Poor cell lysis because of insufficient Proteinase K activity
--Use a fresh or well-stored Proteinase K stock solution.
- Poor cell lysis because of insufficient mixing with FABG buffer
--Mix the sample and FABG 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.
- Ethanol is not added into the lysate before transferring into FABG Midi Column
- Ethanol is not added into Wash Buffer when first open; the volume or the percentage of ethanol is not correct before adding into Wash Buffer.
- Elution of genomic DNA is not efficient
--Make sure the pH of ddH₂O is between 7.5- 8.5.
--After Elution Buffer or ddH₂O is added, stand the FABG Midi Column for 5-10 min before centrifugation.

Column is clogged

- Blood sample contains clots
--Mix the blood sample well with anti-coagulant to prevent formation of blood clots.
- Sample is too viscous
--Reduce the sample volume.

Purified DNA dose not perform well in downstream application

- Sample is old
--Always use fresh or well-stored sample for genomic DNA extraction.
- Residual ethanol contamination
--After Wash step, centrifuge at 4,000 x g for an additional 10 minutes to dry the FABG Midi Column.
- RNA contamination

Special Protocol: (For Bacteria)

Step 1-Sample Preparation

A. For Gram-negative bacteria:

- Transfer the appropriate number of bacterial cell (up to 1×10^9) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 minute. Then discard the supernatant.
- Add 200 μ l of FATG Buffer and resuspend the pellet by vortex or pipetting. Incubate for 5 minutes at room temperature.
- Follow the Cultured Cell Protocol starting from Step 2 (Cell Lysis).

B. For Gram-positive bacteria:

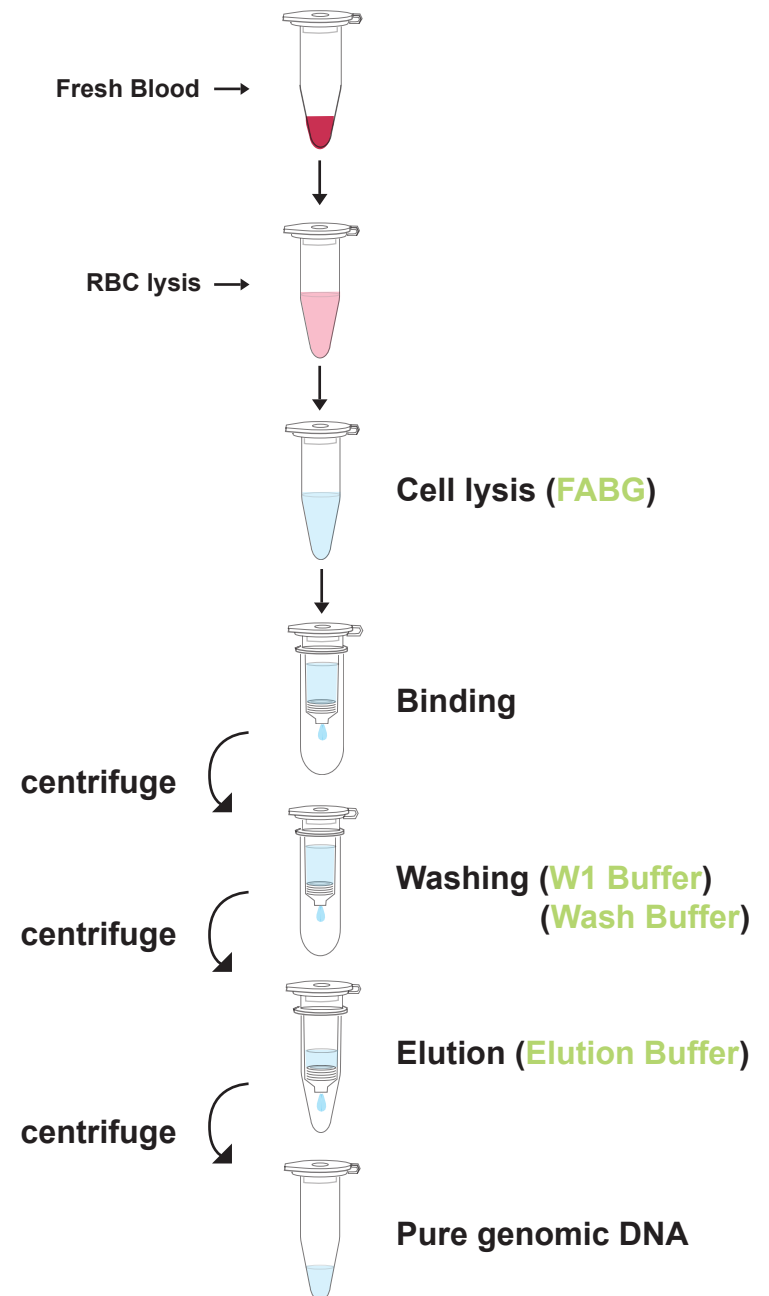
- Transfer the appropriate number of bacterial cell (up to 1×10^9) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 minute. Then discard the supernatant.
- Add 200 μ l of lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100, pH 8.0; prepare fresh lysozyme buffer immediately prior to use) and resuspend the pellet by vortex or pipetting.
- Incubate for 10 minutes at room temperature. During incubation, invert the tube every 2-3 minutes.
- Follow the Cultured Cell Protocol starting from Step 2 (Cell Lysis).

Special Protocol: (For Fungus)

Step 1-Sample Preparation

- Harvest appropriate number of fungus cell (up to 5×10^7) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at 5,000 x g for 10 minute. Then discard the supernatant.
- Add 600 μ l of sorbitol buffer (1.2 M sorbitol; 10 mM CaCl_2 ; 0.1 M Tris-HCl pH 7.5; 35mM β -mercaptoethanol) and resuspend the pellet.
- Add 200 U of lyticase or zymolase. Incubate for 30 minutes at 30°C.
- Centrifuge the mixture at 2,000 x g for 10 minutes to harvest the spheroplast, and then remove the supernatant.

Brief Procedure



General Protocol: (For Fresh Blood)

Please Read Important Notes Before Starting The Following Steps.

Step 1 – RBC Lysis

1. Collect fresh human blood in an anticoagulant-treat collection tube.
2. Transfer up to 300µl fresh blood to a 1.5ml microcentrifuge tube (not provided). If the sample is more than 300 µl (up to 1 ml), add the sample to a sterile 15 ml centrifuge tube.
3. Add 3 x the sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex.
4. Incubate at room temperature for 10 minutes.
5. Centrifuge at 3,000 x g for 5 minutes and completely remove the supernatant.
6. Resuspend the pellet with 100 µl of RBC Lysis Buffer.

Step 2 – Cell Lysis

7. Add 200 µl of FABG Buffer and mix by vortex.
8. Incubate for 10 minutes at room temperature or until the sample lysate is clear. During incubation, invert the tube every 3 minutes.
9. Preheat required Elution Buffer (for Step 5 DNA Elution) in a 70°C water bath.
10. (Optional Step): If RNA-free genomic DNA is required, add 5µl of 10 mg/ml RNase A to the sample and mix by vortex. Then incubate for 5 minutes at room temperature.

Step 3 – Binding

11. Add 200µl ethanol (96~100%) to the sample and vortex for 10 seconds. (Pipetting if there is any precipitate.)
12. Place a FABG Column to a 2ml collection tube. Transfer the sample mixture (including any precipitate) carefully to FABG Column. Centrifuge for 5 minute at full speed (14,000 rpm or 10,000 x g) and discard the 2ml collection tube. Place the FABG Column in a new 2ml Collection tube.

Special Protocol: (For Cultured Cell)

Step 1-Sample Preparation

A. For Cultured Animal Cells:

- i. Trypsinize the adherent cells before harvesting.
- ii. Transfer the appropriate number of cell (up to 1×10^7) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at 6,000 x g for 20 seconds.
- iii. Remove the supernatant and resuspend the cells with 150 µl of RBC Lysis Buffer. Then follow Step 2 (Cell Lysis).

B. For Fresh blood (except human blood):

- i. The sample volume of mammalian blood (non-nucleated) can be up to 50µl; the sample volume of nucleated erythrocytes (eg. bird or fish) can be up to 10µl.
- ii. Add 150 µl of FATG Buffer and the blood sample into a 1.5ml microcentrifuge tube (not provided). Mix by vortex and then follow Step 2 (Cell Lysis).

Step 2 –Cell Lysis

1. Add 200 µl of FABG Buffer to the sample and vortex for 5 seconds.
2. Incubate for 10 minutes at 70°C or until the sample lysate is clear. During incubation, invert the tube every 3 minutes.
3. Preheat required Elution Buffer (for Step 5 DNA Elution) in a 70°C water bath.
4. (Optional Step): If RNA-free genomic DNA is required, add 5µl of 10 mg/ml RNase A to the sample and mix by vortex. Then incubate for 5 minutes at room temperature.
5. Follow the General Protocol starting from Step 3 (Binding).

Step 4 – Binding

11. Add 250µl ethanol (96~100%) to the sample and vortex for 10 seconds. (Pipetting if there is any precipitate.)
12. Place a FABG Column to a 2ml collection tube. Transfer the sample mixture (including any precipitate) to FABG Column. Centrifuge for 5 minute at full speed (14,000 rpm or 10,000 x g) and discard the 2ml collection tube. Place the FABG Column in a new 2ml Collection tube.

Step 5 – Washing

13. Wash FABG Column with 400µl W1 Buffer. Centrifuge for 1 min at full speed (14,000 rpm or 10,000 x g) and discard the flow-through.
14. Place the FABG Column back in the 2ml Collection tube. Wash FABG Column with 600µl Wash Buffer (ethanol added). Centrifuge for 1 min at full speed (14,000 rpm or 10,000 x g) and discard the flow-through.
--Make sure that ethanol has been added into Wash Buffer when first open.
15. Place the FABG Column back in the 2ml Collection tube. Centrifuge for an additional 3 min at full speed (14,000 rpm or 10,000 x g) to dry the column.
--Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

Step 6 – Elution

16. Place the dry FABG Column to a new 1.5ml microcentrifuge tube.
17. Add 100µl of Preheated Elution Buffer or TE to the membrane center of FABG Column.
--Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and absorbed completely.
18. Incubate the FAGB Column at 37°C for 10 minutes in an incubator.
19. Centrifuge for 1 minute at full speed (14,000 rpm or 10,000 x g) to elute the DNA .
--Standard volume for elution is 100 µl. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total volume could be 200 µl.

Step Final - Pure DNA

20. Store the DNA fragment at 4°C or -20°C.

Step 4 – Washing

13. Wash FABG Column with 400µl W1 Buffer. Centrifuge for 30 seconds at full speed (14,000 rpm or 10,000 x g) and discard the flow-through.
14. Place the FABG Column back in the 2ml Collection tube. Wash FABG Column with 600µl Wash Buffer (ethanol added). Centrifuge for 30 seconds at full speed (14,000 rpm or 10,000 x g) and discard the flow-through.
--Make sure that ethanol has been added into Wash Buffer when first open.
15. Place the FABG Column back in the 2ml Collection tube. Centrifuge for an additional 3 min at full speed (14,000 rpm or 10,000 x g) to dry the column.
--Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

Step 5 – Elution

16. Place the dry FABG Column to a new 1.5ml microcentrifuge tube.
17. Add 100µl of Preheated Elution Buffer or TE to the membrane center of FABG Column. Stand FAGB Column for 3~5 min or until the buffer is absorbed by the membrane.
--Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and absorbed completely.
18. Centrifuge for 30 seconds at full speed (14,000 rpm or 10,000 x g) to elute the DNA .
--Standard volume for elution is 100 µl. If sample has low number of cells, reduce the elution volume (30 µl - 50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total volume could be 200 µl.

Step Final - Pure DNA

19. Store the DNA fragment at 4°C or -20°C.

Special Protocol: (For Frozen Blood)

Step 1-Sample Preparation

1. Transfer up to 200µl blood to a 1.5ml microcentrifuge tube (not provided).
If the sample volume is less than 200µl, add the appropriate volume of PBS.
2. Add 30µl Proteinase K (10 mg/ml) to the sample and briefly mix.
Then incubate for 15 minutes at 60°C.

Step 2 –Cell Lysis

3. Add 200µl FABG Buffer to the sample and mix by vortex.
4. Incubate in a 70°C water bath for 15 minutes to lyse the sample.
During incubation, invert the sample every 3 minutes.
5. Preheat required Elution Buffer (for Step 5 DNA Elution) in a 70°C water bath.
6. (Optional Step): If RNA-free genomic DNA is required, add 5µl of 10 mg/ml RNase A to the sample and mix by vortex. Then incubate for 5 minutes at room temperature.
7. Follow the General Protocol starting from Step 3 (Binding).

Special Protocol: (For Buffy Coat)

Step 1-Sample Preparation

Centrifuge whole blood at 3,300xg for 10 minutes at room temperature and you will get three different fractions: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; the bottom layer contains concentrated erythrocytes. Extraction total DNA from buffy coat will yield 5-10 times more DNA than an equivalent volume of whole blood.

Step 2 – RBC Lysis

1. Transfer up to 200µl buffy coat to a 1.5ml microcentrifuge tube (not provided).
2. Add 3 x the sample volume of RBC Lysis Buffer and mix by inversion.
3. Incubate at room temperature for 10 minutes. During incubation, invert the tube every 3 minutes.
4. Centrifuge for 1 minutes at full speed (14,000 rpm or 10,000 x g) and completely remove the supernatant.
5. Resuspend the pellet with 500 µl of RBC Lysis Buffer. Then centrifuge for 1 minutes at full speed (14,000 rpm or 10,000 x g) and completely remove the supernatant.
6. Resuspend the pellet with 200 µl of RBC Lysis Buffer. (Mix the tube by vortex only. Be sure the pellet is completely resuspended or the column would be barred when processing binding step.)

Step 3 – Cell Lysis

7. Add 250 µl of FABG Buffer to the sample and mix by vortex.
8. Incubate for 30 minutes at room temperature or until the sample lysate is clear. During incubation, invert the tube every 3 minutes.
9. Preheat required Elution Buffer (for Step 6 DNA Elution) in a 70°C water bath.
10. (Optional Step): If RNA-free genomic DNA is required, add 5µl of 10 mg/ml RNase A to the sample and mix by vortex. Then incubate for 5 minutes at room temperature.