User Manual

FavorPrep™ Blood/Cultured Cells Genomic DNA Extraction Mini Kit

-For extraction of genomic DNA from fresh blood, forzen blood, cultured cells and fungus

For Research Use Only

Kit Contents:

Cat. No:	FABGK 004 (4 preps)	FABGK 100 (100 preps)	FABGK 300 (300 preps)
RBC Lysis Buffer	7 ml	135 ml	405 ml
FATG Buffer	1.5 ml	30 ml	75 ml
FABG Buffer	1.5 ml × 2	40 ml	100 ml
W1 Buffer	1.8 ml	45 ml	130 ml
Wash Buffer * (concentrate)	1 ml	25 ml	50 ml
Elution Buffer	1 ml	30 ml	75 ml
FABG Mini columns	4 pcs	100 pcs	300 pcs
Collection tubes	8 pcs	200 pcs	600 pcs
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Preparation of **Wash Buffer** by adding ethanol (96~100%)			
* Ethanol volume for Wash Buffer	4 ml	100 ml	200 ml

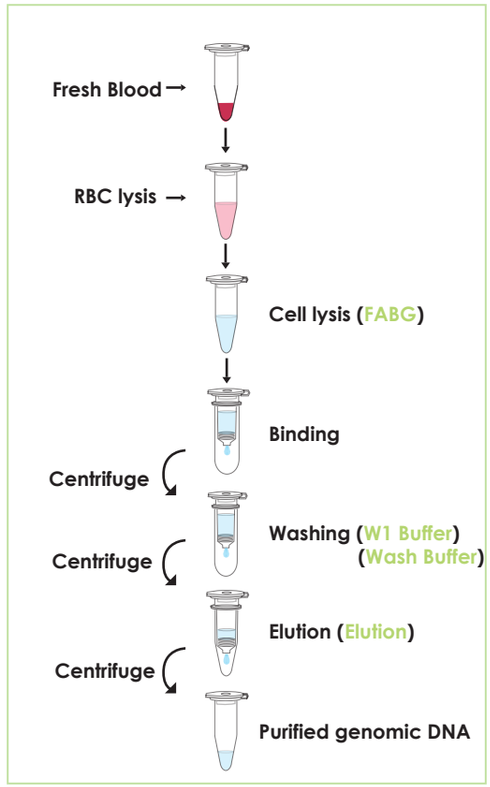
Specification:

Principle: mini spin column (silica matrix)
Operation time: 30~60 minutes
Binding capacity: ≤60 µg DNA/column
Typical yield: 15~35 µg/prep
Column applicability: centrifugation and vaccum
Minimum elution volume: 50 µl
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

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 **Wash Buffer** when first open.
3. Preheat the Elution Buffer to 70°C for step 17.
4. All centrifuge steps are performed at full speed (~18,000 xg) in a microcentrifuge.

Brief procedure:



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General Protocol: Isolation of DNA from Fresh Human Blood

Please Read Important Notes Before Starting Following Steps.

RBC Lysis

1. Collect fresh human blood in an anticoagulant-treat collection tube.
2. Transfer up to 300 µl of fresh blood to a 1.5 ml 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× the sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex.
   For example: add 900 µl of RBC Lysisi Buffer to the 300 µl of the blood sample.
4. Incubate the sample mixture at room temperature for 10 mins.
   -Note: Make sure that the sample mixture become deep-red and transparant after incubation.
5. Centrifuge at 3,000 xg for 5 mins. And completely remove the supernatant.
6. Add 100 µl of RBC Lysis Buffer to the pellet and resuspend the cells by pipetting.

Cell Lysis

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

DNA Binding

11. Add 200 µl of ethanol (96~100%) to the sample and vortex for 10 secs. Pipette the sample to mix well if there is any precipitate formed.
12. Place a FABG Column to a Collection Tube. Transfer the sample mixture carefully to FABG Column. Centrifuge at speed 14,000 rpm or 18,000 xg for 1 min. Discard the Collection Tube and place the FABG Column to a new Collection Tube.

Column Washing

13. Add 400 µl of W1 Buffer to the FABG Column and centrifuge for 30 secs at speed 14,000 rpm or 18,000 xg. Discard the flow-through and place the FABG Column back to the Collection Tube.
14. Add 600 µl of Wash Buffer to the FABG Column and centrifuge for 30 secs at speed 14,000 rpm or 18,000 xg. Discard the flow-through and place the FABG Column back to the Collection Tube.
    -Make sure that ethanol has been added to Wash Buffer when first open.
15. Centrifuge for an additional 3 mins at speed 14,000 rpm or 18,000 xg to dry the column.
    -Important Step! This step will avoid the subsequent enzymatic reactions from being inhibited by residual liquid.

Elution

16. Place the dry FABG Column to a new 1.5 ml microcentrifuge tube.
17. Add 50~200 µl 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.
    -Do not elute the DNA using less than suggested volume (50 µl). It will lower the final yield.
    -If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total volume could be 200 µl.
18. Incubate the FAGB Column at 37°C for 10 mins in an incubator.
19. Centrifuge for 1 min at full speed 14,000 rpm or 18,000 xg to elute the DNA.
20. Store the DNA fragment at 4°C or -20°C.

Protocol: Isolation of DNA from Fresh Non-Human Blood

Please Read Important Notes Before Starting Following Steps.

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

Cell Lysis

1. Add 200 µl of FABG Buffer to the sample and vortex for 5 secs.
2. Incubate the sample mixture 70°C for 10 mins or until the sample mixture is clarified. During incubation, invert the tube every 3 mins.
3. Preheat required Elution Buffer in a 70°C water bath for DNA Elution step.
4. (Optional Step): If RNA-free genomic DNA is required, add 5 µl of 10 mg/ml RNase A to the sample and mix by vortexing. Then incubate for 5 mins at room temperature.
5. Follow the General Protocol starting from Step 11.

Protocol: Isolation of DNA from Frozen Blood

Please Read Important Notes Before Starting Following Steps.

Sample Preparation

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

Cell Lysis

3. Add 200 µl FABG Buffer to the sample and mix by vortexing.
4. Incubate in a 70°C water bath for 15 mins to lyse the sample. During incubation, invert the sample every 3 mins.
5. Preheat required Elution Buffer in a 70°C water bath for DNA Elution.
6. (Optional Step): If RNA-free genomic DNA is required, add 5 µl of 10 mg/ml RNase A to the sample and mix by vortexing. Incubate for 5 min at room temperature.
7. Follow the General Protocol starting from Step 11.

Protocol: Isolation of DNA from Buffy Coat

Please Read Important Notes Before Starting Following Steps.

Sample Preparation

Centrifuge whole blood at 3,300 xg for 10 mins 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.

RBC Lysis

1. Transfer up to 200 µl buffy coat to a 1.5 ml microcentrifuge tube (not provided).
2. Add 3× the sample volume of RBC Lysis Buffer and mix by inversion.
   For example: add 600 µl of RBC Lysisi Buffer to the 200 µl of the buffy coat.
3. Incubate at room temperature for 10 mins. During incubation, invert the tube every 3 mins.
4. Centrifuge for 1 mins at speed 14,000 rpm or 18,000 xg and completely remove the supernatant.
5. Add 500 µl of RBC Lysis Buffer and resuspend the pellet by pipetting. Centrifuge for 1 min at 14,000 rpm or 18,000 xg and completely remove the supernatant.
6. Add 200 µl of RBC Lysis Buffer and resuspend the pellet by vortexing.
   -Note! Make sure the pellet is completely resuspended.

Cell Lysis

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

DNA Binding

11. Add 250 µl of ethanol (96~100%) to the sample and vortex for 10 secs. Pipette the sample to mix well if there is any precipitate formed.
12. Place a FABG Column to a Collection Tube. Transfer the sample mixture carefully to FABG Column. Centrifuge at speed 14,000 rpm or 18,000 xg for 1 min. Discard the Collection Tube and place the FABG Column to a new Collection Tube.
13. Follow the General Protocol starting from Step 13 (**Column Washing**).

Protocol: Isolation of DNA from Cultured Cells

Please Read Important Notes Before Starting Following Steps.

Sample Preparation

i. Trypsinize the adherent cells before harvesting.
ii. Transfer the appropriate number of cells (up to 1×10^7) to a 1.5 ml microcentrifuge tube (not provided) and centrifuge at 6,000 xg for 20 secs.
iii. Remove the supernatant and resuspend the cells with 150 µl of RBC Lysis Buffer.

Cell Lysis

1. Add 200 µl of FABG Buffer to the sample and vortex for 5 secs.
2. Incubate for 10 mins at 70°C or until the sample lysate is clear. During incubation, invert the tube every 3 mins.
3. Preheat required Elution Buffer (for Step **DNA Elution**) in a 70°C water bath.
4. (Optional Step): If RNA-free genomic DNA is required, add 5 µl of 10 mg/ml RNase A to the sample and mix by vortexing. Incubate for 5 mins at room temperature.
5. Follow the General Protocol starting from Step 11.

Protocol: Isolation of DNA from Bacterial cells

Please Read Important Notes Before Starting Following Steps.

Sample Preparation

A. For Gram-negative bacteria:

i. Transfer the appropriate number of bacterial cells (up to 1×10^9) to a 1.5 ml microcentrifuge tube (not provided) and centrifuge at 14,000 rpm or 18,000 xg for 1 min. Discard the supernatant.
ii. Add 200 µl of FATG Buffer and resuspend the pellet by vortexing or pipetting. Incubate for 5 mins at room temperature.
iii. Follow the Cultured Cell Protocol starting from Step 1 (**Cell Lysis**).

B. For Gram-positive bacteria:

i. Transfer the appropriate number of bacterial cell (up to 1×10^9) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at speed 14,000 rpm or 18,000 xg for 1 min. Discard the supernatant.
ii. Add 200 µl 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.
iii. Incubate for 10 mins at room temperature. During incubation, invert the tube every 2~3 mins.
iv. Follow the Cultured Cell Protocol starting from Step 1 (**Cell Lysis**).

Protocol: Isolation of DNA from Fungus

Please Read Important Notes Before Starting Following Steps.

Sample Preparation

i. Harvest appropriate number of fungus cell (up to 5×10^7) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at 5,000 xg for 10 mins. Discard the supernatant.
ii. Add 600 µl of sorbitol buffer (1.2 M sorbitol; 10 mM CaCl; 0.1 M Tris-HCl pH 7.5; 35 mM ß-mercaptoethanol) and resuspend the pellet.
iii. Add 200 U of lyticase or zymolyase. Incubate for 30 mins at 30°C.
iv. Centrifuge the mixture at 2,000 xg for 10 mins to harvest the spheroplast, and then remove the supernatant.
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 mins, and then follow the Cultured Cell Protocol starting from Step 1 (**Cell Lysis**).