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

Kit Contents:

For Research Use Only

Cat. No:	FABGK 004 (4 preps_sample)	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 x 2	40 ml	100 ml
W 1 Buffer	1.3 ml	45 ml	130 ml
Wash Buffer * (concentrate)	1 ml	25 ml	50 ml
Elution Buffer	1 ml	30 ml	75 ml
FABG Mini Column	4 pcs	100 pcs	300 pcs
Collection Tube	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

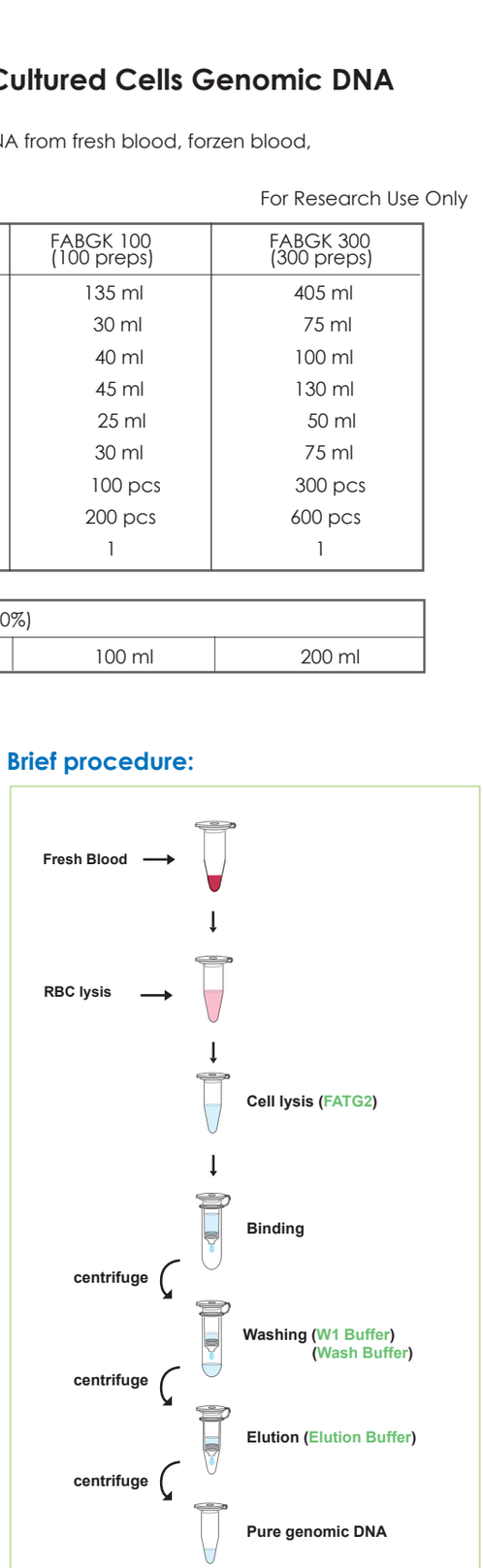
Specification:

Principle: mini spin column (silica matrix)
Operation time: 30 ~ 60 minutes
Binding capacity: up to 50 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 x 10^7 of Cultured animal cells
up to 1 x 10^9 of Cultured bacterial cells
up to 5 x 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 13.
4. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.

Brief procedure:



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 3x 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 min.
   Note: Make sure that the sample mixture become deep-red and transparant after incubation.
5. Centrifuge at 3,000 x g for 5 min. 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. And mix well by vortexing.
8. Incubate the sample mixture at room temperature for 10 min or until the sample mixture is clear. During incubation, invert the tube every 3 min.
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 5 µl of 10 mg/ml RNase A and mix by vortexing. Incubate for 5 min at room temperature.

DNA Binding

11. Add 200 µl of ethanol (96~100%) to the sample and vortex for 10 sec. 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 x g 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 sec at speed 14,000 rpm or 18,000 x g. 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 sec at speed 14,000 rpm or 18,000 x g. 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 min at speed 14,000 rpm or 18,000 x g 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 100 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.
18. Incubate the FAGB Column at 37 °C for 10 min in an incubator.
19. Centrifuge for 1 minute at full speed 14,000 rpm or 18,000 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.
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 seconds.
2. Incubate the sample mixture 70 °C for 10 min or until the sample mixture is clarified. During incubation, invert the tube every 3 min.
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 5µl of 10 mg/ml RNase A to the sample and mix by vortexing. Then incubate for 5 min at room temperature.
5. Follow the General Protocol starting from Step 11 (DNA Binding).

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 1.5ml 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 min 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 min to lyse the sample. During incubation, invert the sample every 3 min.
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 (DNA Binding).

Protocol: Isolation of DNA from Buffy Coat

Please Read Important Notes Before Starting Following Steps.

Sample Preparation

Centrifuge whole blood at 3,300 x g for 10 min 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 x 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 min. During incubation, invert the tube every 3 min.
4. Centrifuge for 1 minutes at speed 14,000 rpm or 18,000 x g 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 x g 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 min at room temperature or until the sample lysate is clear. During incubation, invert the tube every 3 min.
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 5µl of 10 mg/ml RNase A to the sample and mix by vortexing. Then incubate for 5 min at room temperature.

DNA Binding

11. Add 250 µl of ethanol (96~100%) to the sample and vortex for 10 sec. 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 x g 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 x 10^7) to a 1.5 ml 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.

Cell Lysis

1. Add 200 µl of FABG Buffer to the sample and vortex for 5 seconds.
2. Incubate for 10 min at 70 °C or until the sample lysate is clear. During incubation, invert the tube every 3 min.
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 vortexing. Incubate for 5 min at room temperature.
5. Follow the General Protocol starting from Step 11 (DNA Binding).

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 x 10^9) to a 1.5 ml microcentrifuge tube (not provided) and centrifuge at 14,000 rpm or 18,000 x g for 1 min. Discard the supernatant.
ii. Add 200 µl of FATG Buffer and resuspend the pellet by vortexing or pipetting. Incubate for 5 min 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 x 10^9) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at speed 14,000 rpm or 18,000 x g 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 minutes at room temperature. During incubation, invert the tube every 2-3 min.
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 x 10^7) to a 1.5ml microcentrifuge tube (not provided) and centrifuge at 5,000 x g for 10 min. 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; 35mM ß-mercaptoethanol) and resuspend the pellet.
iii. Add 200 U of lyticase or zymolase. Incubate for 30 min at 30°C.
iv. Centrifuge the mixture at 2,000 x g for 10 min 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 min, and then follow the Cultured Cell Protocol starting from Step 1 (Cell Lysis).