FavorPrep™
Blood / Cultured Cell Genomic DNA Extraction Mini Kit

User Manual

Cat. No.: FABGK 001 (50 Preps)
FABGK 001-1 (100 Preps)
FABGK 001-2 (300 Preps)

For Research Use Only

v.1002

Introduction

FavorPrep™ Genomic DNA Extraction Mini Kit is an excellent tool offering a speedy and economic method to purify total DNA (e.g. genomic, mitochondrial and viral DNA) from whole blood (fresh or frozen), plasma, serum, buffy coat, body fluids, lymphocytes and cultured cells. This technology first lyses cells and degrades protein by using a chaotropic salt and Proteinase K, then binds DNA to silica-based membranes, washes DNA with ethanol-contained Wash Buffer and then elutes purified DNA by low salt Elution Buffer or ddH_2O. Compare with other harmful and time-consuming procedures, such as phenol/chloroform extraction and ethanol precipitation, FavorPrep™ shortens the handling time within 1 hour. The size of purified DNA is up to 50 Kb (predominantly 20 - 30 Kb). After using FavorPrep™ Genomic DNA Extraction Mini Kit, the high quality total DNA can be used directly for the downstream applications.

Specification

Sampling: up to 200 µl of whole blood (with anti-coagulant), plasma, serum, buffy coat, or body fluids; up to 5 x 10^6 lymphocytes or cultured cells in 200 µl PBS.

Yield: about 5 µg of total DNA from 200 µl of human whole blood; up to 50 µg of total DNA, depends on the sample types and the number of cells in the sample.

Handling time: within 1 hour, depends on the sample types.

Kit Contents

	FABGK001 (50preps)	FABGK001-1 (100preps)	FABGK001-2 (300preps)
FABG Buffer	15 ml	30 ml	70 ml
W1 Buffer*	22 ml	44 ml	124 ml
Wash Buffer**	10 ml	20 ml	50 ml
Elution Buffer	15 ml	30 ml	30 ml x 3
Proteinase K***	11 mg	11 mg x 2	11 mg x 6
FABG Mini Column	50 pcs	100 pcs	300 pcs
Collection Tube	100 pcs	200 pcs	600 pcs
Elution Tube	50 pcs	100 pcs	300 pcs

*Add 8 / 16 / 45 ml ethanol (96~100%) to W1 Buffer when first open.

**Add 40 /80 / 200 ml ethanol (96~100%) to Wash Buffer when first open.

***Add 1.1 ml sterile ddH_2O to each Proteinase K tube to make a 10mg/ml stock solution.

Important Notes

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.

2. Add 1.1 ml sterile ddH_2O to each Proteinase K tube to make a 10mg/ml stock solution. Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4°C**.

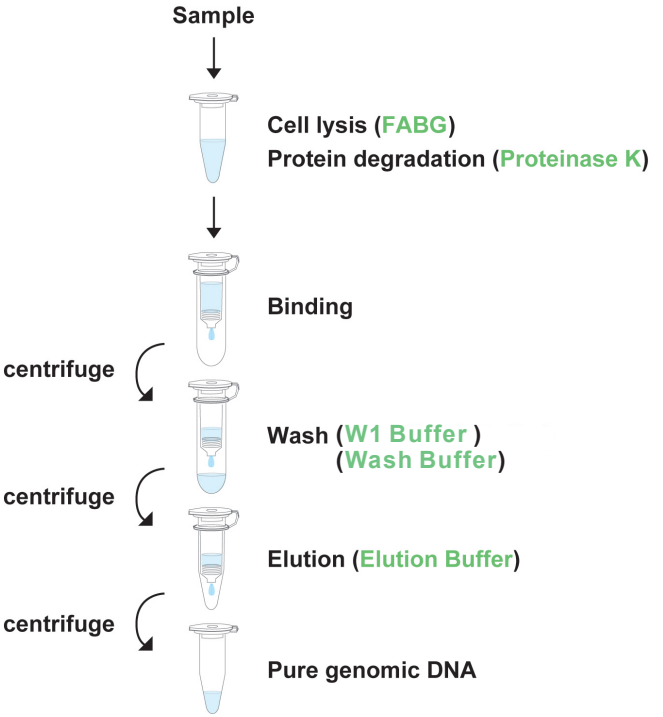
3. For FABGK 001(50preps), add 8ml ethanol (96~100%) to W1 Buffer when first open. For FABGK 001-1 (100presp), add 16 ml ethanol (96~100%) to W1 Buffer when first open. For FABGK 001-2 (300presp), add 45 ml ethanol (96~100%) to W1 Buffer when first open.

4. For FABGK001 (50preps), add 40ml ethanol (96~100%) to Wash Buffer when first open. For FABGK 001-1 (100preps), add 80 ml ethanol (96~100%) to Wash Buffer when first open. For FABGK 001-2 (300preps), add 200 ml ethanol (96~100%) to Wash Buffer when first open.

5. Preheat a dry bath or a water bath to 60°C before the operation.

6. All centrifuge steps are done at full speed (14,000 rpm or 10,000 xg) in a microcentrifuge.

Brief Procedure



General Protocol

Please Read Important Notes Before Starting The Following Steps.
HINTP: Preheat a 60°C dry bath or water bath for step 4.

1.Transfer up to 200 µl sample (whole blood, buffy coat) to a micropcentrifuge tube (not provided).

・If the sample volume is less than 200 µl, add the appropriate volume of PBS.

2. (Optional): If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A to the sample and incubate for 2 minutes at room temperature.

3. Add 20 µl Proteinase K and 200 µl FABG Buffer to the sample. Mix thoroughly by pulse-vortexing.

・Do not add Proteinase K directly to FABG Buffer.

4. Incubate at 60°C for 15 minutes to lyse the sample. During incubation, vortex the sample every 3 - 5 minutes.

5. Briefly spin the tube to remove drops from the inside of the lid.

6. Add 200 µl ethanol (96~100%) to the sample. Mix thoroughly by pulse-vortexing for 30 seconds.

7. Briefly spin the tube to remove drops from the inside of the lid.

8. Place a FABG Column to a collection tube. Transfer the sample mixture (including any precipitate) carefully to FABG Column. Centrifuge for 1 minute and discard the flow-through then place FABG Column to a new Collection tube.

9. Immediately, Wash FABG Column with 500 µl W1 Buffer by centrifugefor 1 minute then discard the flow-through.

- Make sure that ethanol has been added into W1 Buffer when first open.

10. Wash FABG Column with 750 µl Wash Buffer by centrifuge for 1 minute then discard the flow-through.

- Make sure that ethanol has been added into Wash Buffer when first open.

11. Centrifuge for an additional 3 min to dry the column.

- **Important Step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

12. Place FABG Column to Elution Tube.

13. Add 100~200 µl of Elution Buffer or ddH_2O (pH 7.5~9.0) to the membrane center of FABG Column. Stand FAGB Column for 3 min.

- **Important Step!** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
- Standard volume for elution is 200 µl, If sample has low number of cells, reduce the elution volume (50µl - 150µl) to increase DNA concert ration.

14. Centrifuge for 2 min to elute the DNA .

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

Special Protocol

For cultured Cells

1. Harvest Cells

a. Cells grown in suspension

i. Transfer the appropriate number of cell (up to 5 x 10^6) to a 1.5ml microcentrifuge tube (not provided).

ii. Centrifuge at 300 x g for 5 minutes.

iii. Remove the supernatant carefully and completely.

b. Cells grown in monolayer

i. Detach cells from the dish or flask by trypsinization or using a cell scraper.

ii. Transfer the appropriate number of cell (up to 5 x 10^6) to a 1.5ml microcentrifuge tube (not provided).

iii. Centrifuge at 300 x g for 5 minutes.

iv. Remove the supernatant carefully and completely.

2. Resuspend cell pellet in PBS to a final volume of 200µl.

3. Follow the General Protocol starting from step 2.

Preparation of buffy coat

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. Process the General Protocol from Step 1 for buffy coat. Extraction total DNA from buffy coat will yield 5-10 times more DNA than an equivalent volume of whole blood.

Trouble Shooting

Problem	Possible Reasons/ Solution
Low or no yield of genomic DNA	**1. Low amount of cells in the sample** ・Concentrate a larger volume of a new sample to 200 µl. If the sample is whole blood, prepare buffy coat (refer to Special Protocol on page 7).
	2. Poor cell lysis **A. it is because of insufficient Proteinase K activity** ・Repeat the extraction procedure with a new sample. Use a fresh or well-stored proteinase K stock solution. **B. it is because of insufficient mixing with FABG Buffer** ・Repeat the extraction procedure with a new sample. Mix the sample and FABG Buffer immediately and thoroughly by pulse-vortexing. **C. It is because of insufficient incubation time.** ・Repeat the extraction procedure with a new sample. Extend the incubation time and make sure that no residual particulates remain.
	3. Ethanol is not added into the lysate before transferring sample mixture into FABG Column ・Repeat the extraction procedure with a new sample.
	4. Incorrect preparation of Wash Buffer **A. Ethanol is not added into Wash Buffer when first open.** ・Make sure that the correct volumes of ethanol (96~100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample.

Trouble Shooting

Problem	Possible Reasons/ Solution
Low or no yield of genomic DNA	**B. The volume or the percentage of ethanol is not correct before adding into Wash Buffer** ・Make sure that the correct volumes of ethanol (96~100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample. **5. Elution of genomic DNA is not efficient** **A. PH of water (ddH_2O) for elution is acidic** ・Make sure the pH of ddH_2O is between 7.5~9.0. ・Use Elution Buffer (provided) for elution. **B. Elution Buffer or ddH_2O is not completely absorbed by column membrane** ・After Elution Buffer or ddH_2O is added, stand the FABG Column for 5 minutes before centrifugation.
Brown residues remain on the column membrane after washing	**1. Poor Cell Lysis** **A. It is because of insufficient Proteinase K activity** ・Repeat the extraction procedure with a new sample. Use a fresh or well-stored Proteinase K stock solution. ・Do not add Proteinase K stock directly to FABG Buffer. **B. It is because of insufficient mixing with FABG Buffer** ・Repeat the extraction procedure with a new sample. Mix the sample and FABG Buffer immediately and thoroughly by pulse-vortexing. **C. Poor cell lysis because of insufficient incubation time** ・Repeat the extraction procedure with a new sample. Extend the incubation time and make sure that no residual particulates remain.

Trouble Shooting

Problem	Possible Reasons/ Solution
Brown residues remain on the column membrane after washing	**2. Ethanol is not added into the lysate before transferring sample mixture into FABG Column** ・Repeat the extraction procedure with a new sample.
	3. Incorrect preparation of Wash Buffer **A. Ethanol is not added into Wash Buffer when first open** ・Make sure that the correct volumnes of ethanol (96~100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample. **B. The volumn or the percentage of ethanol is not correct before adding into Wash Buffer** ・Make sure that the correct volumnes of ethanol (96~100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample.
Column is clogged	**1. Blood sample contains clots** ・Repeat the extraction procedure with a new sample. Mix the blood sample well with anticoagulant to prevent formation of blood clots. **2. Sample is too viscous** ・Reduce the sample volume. **3. Insufficient activity of Proteinase K** ・Use a fresh or well-stored Proteinase K Stock solution.. ・Repeat the extraction procedure with a new sample. Do not add Proteinase K Into FABG Buffer directly.

Trouble Shooting

Problem	Possible Reasons/ Solution
A_{280}/A_{280} ratio of eluted DNA is low	**Poor cells lysis**
	1. Poor cell lysis because of insufficient Proteinase K activity ・Repeat the extraction procedure with a new sample. Use a fresh or well-stored Proteinase K stock solution. ・Do not add Proteinase K directly to FABG Buffer.
	2. Poor cell lysis because of insufficient mixing with FABG Buffer ・Repeat the extraction procedure with a new sample. Mix the sample and FABG Buffer immediately and throughly by pulse-vortexing.
	3. Poor cell lysis because of insufficient incubation time ・Repeat the extraction procedure with a new sample. Extend the incubation time and make sure that no residual particulates remain.
	Ethanol is not added into the lysate before transferring sample mixture into FABG Mini Column ・Repeat the extraction procedure with a new sample.
	Incorrect preparation of Wash Buffer
	4. Ethanol is not added into Wash Buffer when first open ・Make sure that the correct volumes of ethanol (96 - 100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample.

Trouble Shooting

Problem	Possible Reasons/ Solution
A_{280}/A_{280} ratio of eluted DNA is low	**5. The volume or the percentage of ethanol is not correct before adding into Wash Buffer** ・Make sure that the correct volumes of ethanol (96 - 100%) is added into Wash Buffer when first open. Repeat the extraction procedure with a new sample.
	6. Genomic DNA is contaminated ・Do not wet the rim of the column during sample and buffer loading.
A_{280}/A_{280} ratio of eluted DNA is high	**1. A lot of residual RNA in eluted DNA** ・Follow the General Protocol step 2 to remove RNA.
	2. FABG Buffer added to the sample before adding RNase A ・Make sure that Rnase A has been added to the sample before adding FABG Buffer when using optional RNase step.
Degradation of eluted DNA	**1. Sample is old** ・Always use fresh or well-stored sample for genomic DNA extraction.
	2. Buffer for gel electrophoresis contaminated with DNase ・Use fresh running buffer for gel electrophoresis.

Storage Conditions

FavorPrep™ Genomic DNA Extraction Mini Kit except Proteinase K can be stored at room temperature 15 - 25°C for up to 1 year. Proteinase K powder can be stored dry at room temperature for up to 6 months. For storage longer than 6 months Proteinase K powder should be stored dry at 2-8°C. Proteinase K stock solution is stable for 2 months when stored at 2-8°C. Storage at -20°C will prolong its life, but repeated freezing and thawing should be avoided.