FavorPrep™ Blood/ Cultured Cell Genomic DNA Extraction Midi Kit

User Manual

For Research Use Only

Cat. No.: FABGK 002 (20 Preps)
FABGK 002-1 (50 Preps)

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 Midi Kit, the high quality total DNA can be used directly for the downstream applications.

Kit Contents

Cat. No. / preps	FABGK 002 (20 Preps)	FABGK 002-1 (50 Preps)
Proteinase K powder	16.5 mg	41.5 mg
FABG Buffer	35 ml	85 ml
W1 Buffer* (concentrated)	33 ml	88 ml
Wash Buffer** (concentrated)	20 ml	40 ml
Elution Buffer	30 ml	65 ml
FABG Midi Column	20 pcs	50 pcs
Elution Tube (50 ml tube)	20 pcs	50 pcs
User Manual	1	1

*Add 12 ml / 32 ml of ethanol (96~100%) to W1 Buffer when first open.

**Add 80 ml / 160 ml of ethanol (96~100%) to Wash Buffer when first open.

Specification

Sample Size : up to 1.5 ml of fresh/ frozen blood
up to 6^{7} x 10 of cultured cells

Column Capacity: 200 µg of DNA

Average DNA yield : 35 µg/ 1 ml whole blood

Format: spin column

Handling Time: 1 hours

Elution Volume: 1 ml

Important Notes

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

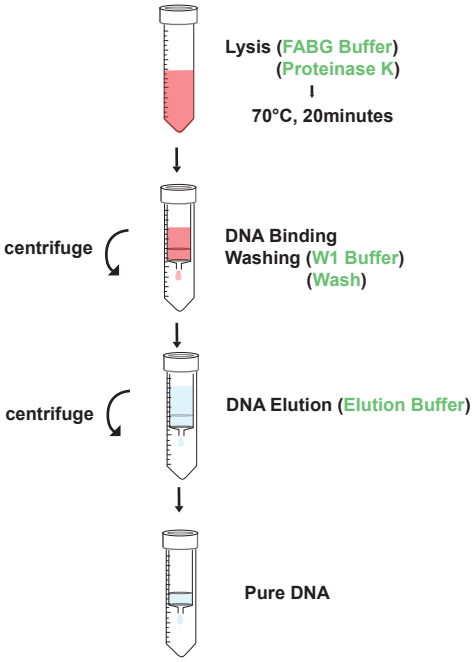
2. **For Cat. No. FABGK 002, add 1.65 ml of sterile ddH2O to Proteinase K tube; For Cat. No. FABGK 002-1, add 4.05 ml of sterile ddH2O to proteniase K tube to make a 10 mg/ ml stock solution. Vortex and make sure that Proteinase K powder has been completely dissolved. Store the stock solution at 4 °C.**

3. **For Cat. No. FABGK 002, add 12 ml of ethanol (96~100 %) to W1 Buffer when first open. For Cat. No. FABGK 002-1, add 32 ml of ethanol (96~100%) to W1 Buffer when first open.**

4. **For Cat. No. FABGK 002, add 80 ml of ethanol (96~100%) to Wash Buffer when first open. For Cat. No. FABGK002-1, add 160 ml of ethanol (96~100%) to wash Buffer when first open.**

5. **Preheat a dry bath or water bath to 70 °C before the operation.**

Brief Procedure



Protocol: (for Blood DNA Extraction)

Please Read Important Notes Before Starting The Following Steps.

1. Transfer up to 1.5 ml sample (whole blood, buffy coat) to a 15 ml centrifuge tube (not provided).
   ---If lymphocytes sample, transfer 10^7~10^8 cells to a 15 ml centrifuge tube and make total volume to 1 ml with PBS.

2. Add 75 µl of Proteinase K (10 mg/ml) to the sample, mix well by vortexing. And then add 1.5 ml of FABG Buffer to the sample mixture, mix thoroughly by pulse-vortexing.
   ---Do not add Proteinase K directly to FABG Buffer.

3. Incubate the sample mixture at 70 °C for 20 minutes to lyse the sample. During incubation, invert the tube every 3-5 minutes.
   ---At this time, preheat required Elution Buffer or ddH_2O (1ml per sample) to 70 °C. (For DNA Elution step)

4. **(*Optional*): If RNA-free genomic DNA is required, add 2 µl of 100 mg/ml RNase A (not provided) to the sample mixture and incubate at room temperature for 5 minutes.**

5. **Add 1.5 ml of ethanol (96- 100 %) to the sample mixture. Mix thoroughly by vortexing. If precipitate appears, break it by pipetting.**

6. **Place a FABG Midi Column to a 15 ml centrifuge tube (not provided). And transfer total sample mixture (ethanol added) (including any precipitate) carefully to the FABG Midi Column. Close the cap and centrifuge at 4,000 x g for 3 min.**

7. **Add 2.0 ml of W1 Buffer (ethanol added) to the FABG Midi Column. Close the cap and centrifuge at 4,000 x g for 3 min. Discard the flow-through and place the FABG Midi Column back in the 15 ml centrifuge tube.**
   ---Make sure that ethanol has been added into W1 Buffer when first open.

8. **Add 3.5 ml of Wash Buffer (ethanol added) to the FABG Midi Column. Close the cap and centrifuge at 4,000 x g for 3 min. Discard the flow-through and place the FABG Midi Column back in the 15 ml centrifuge tube.**
   ---Make sure that ethanol has been added into Wash Buffer when first open.

9. **Centrifuge at 4,000 x g for an additional 10 min to dry the column.**
   ---*Important Step!* The residual liquid can affect the quality of DNA and inhibit subsequent enzymatic reactions.

10. **Place the FABG Midi Column into a new 15 ml centrifuge tube (Elution Tube). (provided)**

11. **Add 1 ml of preheat Elution Buffer or ddH_2O (pH 7.5- 9.0) to the membrane center of FABG Midi Column. Stand FABG Midi Column for 5 min at room temperature.**
    ---*Important Step!* For effective elution, stand the FABG Midi Column for 5 minutes is required to make sure that Elution Buffer is absorbed completely by column membrane.

12. **Centrifuge at 4,000 x g for 2 minutes to elute total DNA.**

Protocol: (for Cultured Cell DNA Extraction)

Please Read Important Notes Before Starting The Following Steps.

1. Transfer up to 6 x 10^7 of cells to a 15 ml centrifuge tube (not provided). Centrifuge at 4,000 x g for 5 minutes to pellet the cells.
   ---If using adherent cells, trypsinize the cells before harvesting.

2. Resuspend the cells with 1.5 ml of PBS.

3. Add 75 µl of Proteinase K (10 mg/ml) to the sample. Mix thoroughly by vortexing.

4. Add 1.5 ml of FABG Buffer to the sample mixture. Mix thoroughly by Pulse -vortexing.
   ---Do not add Proteinase K directly to FABG Buffer.

5. Incubate the sample mixture at 70 °C for 20 minutes to lyse the sample. During incubation, invert the tube every 3-5 minutes.
   ---At this time, preheat required Elution Buffer or ddH_2O(1ml per sample) to 70 °C. (For DNA Elution)

6. Follow the Blood protocol starting from step 4. (page 4)

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 ddH_2O is between 7.5- 8.5.
  --- After Elution Buffer or ddH_2O 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