FavorPrep™ Blood/Cultured Cells Genomic DNA Extraction Midi Kit

(For Research Use Only)

Kit Contents:

Cat. No. (preps)	FABGK002-S (2 preps)	FABGK002 (25 preps)
Proteinase K (Liquid)	450 µl	1050 µl × 4
FABG Buffer	4 ml	42 ml
W1 Buffer * (Concentrate)	2.75 ml	44 ml
Wash Buffer ** (Concentrate)	2 ml	25 ml
Elution Buffer	2.2 ml	30 ml
FABG Midi Column	2 pcs	25 pcs
Elution Tube (15 ml tube)	2 pcs	25 pcs
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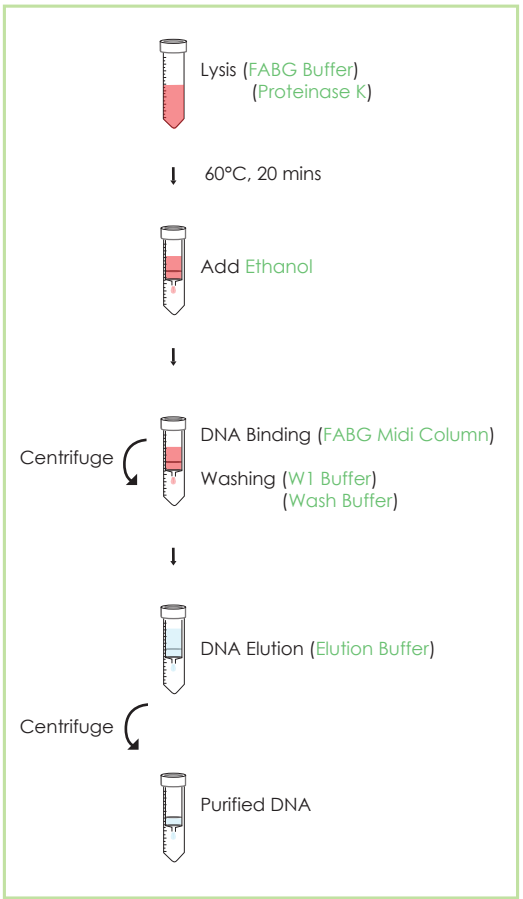
Preparation of W1 Buffer and Wash Buffer for the first use:

Cat. No:	FABGK002-S (2 preps)	FABGK002 (25 preps)
* Ethanol volume for W1 Buffer	1 ml	16 ml
** Ethanol volume for Wash Buffer	8 ml	100 ml

Specification:

Principle: spin column (silica membrane)
Sample Size : up to 1.5 ml of fresh/frozen blood;
up to 6×10^7 of cultured cells.
Column Capacity: 150 µg of DNA
Average DNA yield : 35 µg/ml of whole blood
Handling Time: 1 hr
Elution Volume: 1 ml



Required material to be provided by user

- Pipettors and pipet tips
- Centrifuge (should be capable up to 4,500 x g)
- Thermal incubator
- Oven (optional)
- Ethanol (96~100%)
- Vortex

Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Preheat a thermal incubator to 60°C and 70°C before the operation.
3. Use a centrifuge with a swinging bucket rotor and a force of 4,500~6,000 x g for in all centrifugation steps.
4. Add ethanol (96~100%) to W1 Buffer and Wash Buffer before first use and store at room temperature.

General Protocol: For Whole 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 tube (not provided).
   -If lymphocytes sample is used, transfer 10^7~10^8 cells to a 15 ml tube and adjust total volume to 1 ml with PBS.

2. Add 150 µl of Proteinase K to the sample and mix well by vortexing.

3. Add 1.5 ml of FABG Buffer to the sample and mix thoroughly by vortexing. Incubate the sample mixture at 60°C for 20 mins. During incubation, vortex briefly the tube 3 times and preheat Elution Buffer or ddH_2O (0.5~1 ml per preparation) to 70°C.
   -Do not add Proteinase K directly to FABG Buffer.

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

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

6. Place a FABG Midi Column to a 15 ml centrifuge tube (not provided). Transfer total sample mixture (ethanol added) carefully to the FABG Midi Column. Close the cap and centrifuge at 4,500~6,000 x g for 3 mins. Discard the flow-through and place the FABG Midi Column back to the 15 ml centrifuge tube.

7. Add 2 ml of W1 Buffer (ethanol added) to the FABG Midi Column. Close the cap and centrifuge at 4,500~6,000 x g for 3 mins. Discard the flow-through and place the FABG Midi Column back to the 15 ml centrifuge tube.
   -Make sure that ethanol has been added into W1 Buffer at the first use.

8. Add 4.5 ml of Wash Buffer (ethanol added) to the FABG Midi Column. Close the cap and centrifuge at 4,500~6,000 x g for 10 mins. Discard the 15 ml centrifuge tube and the flow- through.
   -Make sure that ethanol has been added into Wash Buffer at the first use.
   -Avoid column tip touch the flow-through when transferring the FABG Midi Column.
   -Note! 10 mins centrifugation is important for removing the residual of Wash Buffer from column membrane.

9. Transfer the FABG Midi Column to a new 15 ml centrifuge tube (Elution Tube, provided). Do not close the cap and stand the column at room temperature for 5 mins.

10. Add 0.5~1 ml of preheat Elution Buffer or ddH_2O (pH 7.5-9.0) to the membrane center of FABG Midi Column. Stand the FABG Midi Column for 2 mins at room temperature.
    -Important Step! Make sure that Elution Buffer is absorbed completely by column membrane.

11. Close the cap and centrifuge at 4,500~6,000 x g for 3 mins to elute DNA.

Protocol: For Cultured Cell DNA Extraction

Please Read Important Notes Before Starting the Following Steps.

1. Transfer up to 6×10^7 of cells to a 15 ml centrifuge tube (not provided). Centrifuge at 4,500 x g for 5 mins to pellet the cells.
   - If using adherent cells, trypsinize the cells before harvesting.

2. Resuspend the cells with 1.5 ml of PBS. Add 150 µl of Proteinase K to the sample and mix well by vortexing.

3. Add 1.5 ml of FABG Buffer to the sample mixture and mix thoroughly by vortexing. Incubate the sample mixture at 60°C for 20 mins to lyse the sample. During incubation, invert the tube every 3~5 mins and preheat Elution Buffer or ddH_2O (0.5~1 ml per preparation) to 70°C.
   - Do not add Proteinase K directly to FABG Buffer.

4. Follow the Whole Blood DNA Extraction protocol starting from step 4.