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 powder ■	3.5 mg	11 mg x 4 tubes
FABG Buffer	4 ml	42 ml
Wash Buffer W1 * (concentrate)	2.75 ml	44 ml
Wash Buffer W2 ** (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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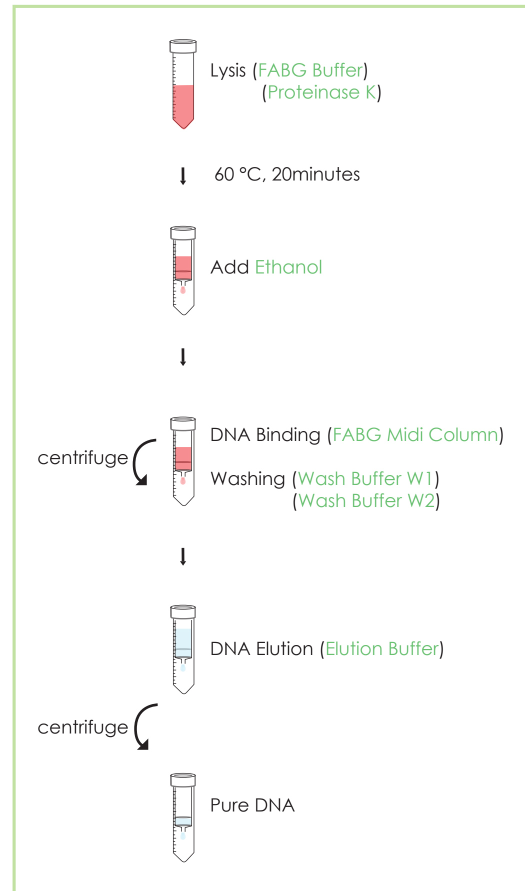
Preparation of ProteinaseK solution (10mg/ml) and Wash Buffer for first use:

Cat. No:	FABGK002-S (2 preps)	FABGK002 (25 preps)
■ ddH2O volume for Proteinase K	0.35 ml	1.1 ml/ tube
* ethanol volume for Wash Buffer W1	1 ml	16 ml
** ethanol volume for Wash Buffer W2	8 ml	100 ml

Specification:

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



Required material to be provided by user

- Pipettors and pipet tip
- Centrifuge (should be capable of producing a force of 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 65 °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 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.
5. Add ethanol (96~100 %) to Wash W1 Buffer and Wash W2 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, transfer 10^{7}~10^{8} cells to a 15 ml tube and make total volume to 1 ml with PBS.

2. Add 150 µl of Proteinase K (10 mg/ml) 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 min. During incubation, vortex briefly the tube 3 times and preheat Elution Buffer or ddH_2O (0.5 ~1ml per preparation) to 70 °C. (For DNA Elution step).
   -- 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 minutes.

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 min. Discard the flow-through and place the FABG Midi Column back to the 15 ml centrifuge tube.

7. Add 2 ml of Wash Buffer W1 (ethanol added) to the FABG Midi Column. Close the cap and centrifuge at 4,500 ~ 6,000 x g for 3 min. 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 Wash Buffer W1 when first use.

8. Add 4.5 ml of Wash Buffer W2 (ethanol added) to the FABG Midi Column. Close the cap and centrifuge at 4,500 ~ 6,000 x g for 10 min. Discard the 15 ml centrifuge tube and the flow- through.
   -- Make sure that ethanol has been added into Wash Buffer W2 when first use.
   -- Do not let the column tip touch the flow-through when transferring the FABG Midi Column.
   -- Note ! 10 min 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 min .

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 min 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 minutes to elute 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 4,500 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. Add 150 µl of Proteinase K (10 mg/ml) 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 minutes to lyse the sample. During incubation, invert the tube every 3-5 minutes and preheat Elution Buffer or ddH_2O (0.5 ~1ml per preparation) to 70 °C. (For DNA Elution step).
   -- Do not add Proteinase K directly to FABG Buffer.

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