FavorPrep™ Blood / Cultured Cell Genomic DNA Extraction Maxi Kit

(For Research Use Only)

Kit Contents:

Cat. No. / preps	FABGK000-Maxi (2 preps)	FABGK003 (10 preps)	FABGK003-1 (24 preps)
Proteinase K powder+	11 mg x 2	11 mg x 10	11 mg x 24
FABG Buffer	22 ml	110 ml	265 ml
W1 Buffer* (concentrate)	6.5 ml	33 ml	88 ml
Wash Buffer** (concentrate)	3 ml	20 ml	40 ml
Elution Buffer	6 ml	30 ml	60 ml
FABG Maxi Column	2 pcs	10 pcs	24 pcs
Elution Tube (50 ml tube)	2 pcs	10 pcs	24 pcs
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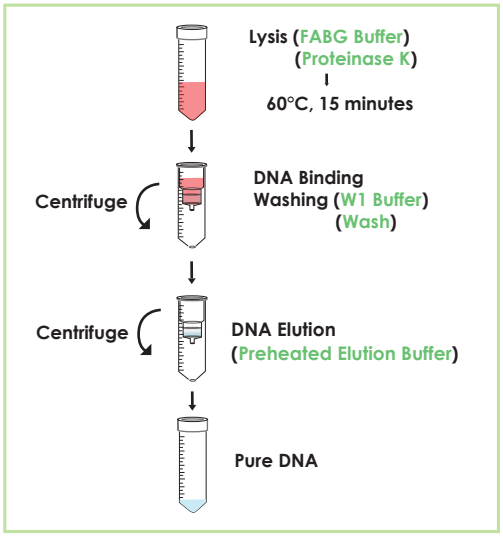
Preparation of ProteinaseK solution (20mg/ml), W1 Buffer and Wash Buffer for first use:

Cat. No:	FABGK000-Maxi (2 preps)	FABGK003 (10 preps)	FABGK003-1 (24 preps)
+ ddH2O volume for Proteinase K	0.5 ml		
* ethanol volume for W1 Buffer	2.5 ml	12 ml	32 ml
**ethanol volume for Wash Buffer	12 ml	80 ml	160 ml

Specification:

Principle: spin column (silica membrane)
Sample Size: up to 10 ml of fresh/ frozen blood;
up to 1 x 10^8 of cultured cells
Column Capacity: 500 µg of DNA
Average DNA yield: 35 µg/1 ml whole blood
Handling Time: 1 hour
Elution Volume: 0.75~1.5 ml



Required material to be provided by user

Pipettors and pipet tip
Centrifuge: should be capable of producing a force of 4,000 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 before the operation.
3. Use a centrifuge with a swinging bucket rotor and a force of 4,000~6,000 x g for in all centrifugation steps.
4. Add 500 µl of sterile ddH2O to proteinase K tube to make a 22 mg/ ml stock solution. Vortex and make sure that Proteinase K powder has been completely dissolved. Store the stock solution at 4°C.
5. Preheat the Elution Buffer or ddH2O for step 11. (Elution step).

Protocol: For Blood DNA Extraction

Please Read Important Notes Before Starting the Following Steps.

1. Transfer up to 10 ml sample (whole blood, buffy coat) to a 50 ml centrifuge tube (not provided). If the sample volume is less than 10 ml, add PBS to make volume to 10 ml.
2. Add 500 µl of Proteinase K (22 mg/ml) to the sample and mix well by vortexing. Add 10 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 60°C for 15 min to lyse the sample. During incubation, invert the tube every 3-5 minutes.
4. **(Optional)**: If RNA-free genomic DNA is required, add 80 µl of 100 mg/ml RNase A (not provided) to the sample mixture and incubate at room temperature for 10 minutes.
5. Add 10 ml of ethanol (96~100%) to the sample mixture. **Mix thoroughly by vortexing. If precipitate appears, break it by pipetting.**
6. Place a FABG Maxi Column to a 50 ml centrifuge tube (not provided). And transfer 15 ml of sample mixture (ethanol added) (including any precipitate) carefully to the FABG Maxi Column. Close the cap and **centrifuge at 4,000~6,000 x g for 3 min**.

7. Discard the flow-through and transfer the rest sample mixture to the same FABG Maxi Column. Close the cap and **centrifuge at 4,000~6,000 x g for 3 min** and discard the flow-through.
8 Add 4 ml of W1 Buffer (ethanol added) to the FABG Maxi Column. Close the cap and **centrifuge at 4,000~6,000 x g for 3 min**. Discard the flow-through and place the FABG Maxi Column back in the 50 ml centrifuge tube.
   --- Make sure that ethanol has been added into W1 Buffer when first open.
9. Add 7 ml of Wash Buffer (ethanol added) to the FABG Maxi Column. Close the cap and **centrifuge at 4,000~6,000 x g for 15 min**. Discard the flow-through and place the FABG Maxi Column back in the 50 ml centrifuge tube.
   --- Make sure that ethanol has been added into Wash Buffer when first open.
   --- **Important Step!** Make sure the residual liquid will be removed completely after centrifugation. It might be necessary to do a further drying by placing the column in a vacuum oven at 70°C for 3 minutes.
10. Place the FABG Maxi Column into a new 50 ml centrifuge tube. (Elution Tube, provided)
11. Add 0.75~1.5 ml of preheat Elution Buffer or ddH_2O (pH 7.5- 9.0) to the membrane center of the FABG Maxi Column. **Stand the FABG Maxi Column for 5 min at room temperature.**
   --- **Important Step!** For effective elution, stand the FABG Maxi Column for 5 minutes is required to make sure Elution Buffer is absorbed completely by column membrane.
   --- Standard volume for elution is 1 ml. If higher DNA yield is required, repeat the DNA Elution step (step 11.) to increase DNA recovery.
12. **Centrifuge at 4,000 x g for 2 minutes** to elute total DNA.

Protocol: For Cultured Cell DNA Extraction

1. Transfer up to 1 x 10^8 of cells to a 50 ml centrifuge tube (not provided). **Centrifuge at 4,000~6,000 x g for 5 minutes** to pellet the cells. (If using adherent cells, trypsinize the cells before harvesting.)
2. Resuspend the cells with 10 ml of PBS.
3. Follow the Blood protocol starting from step 2.

Troubleshooting

Possible reasons	Solutions
Low or no yield of genomic DNA	
Poor cell lysis	
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.
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 thoroughly by pulse-vortexing.
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 into FABG Maxi Column	Repeat the extraction procedure with a new sample.
Incorrect preparation of Wash Buffer	
Ethanol is not added into W1 and Wash Buffer when first open	Make sure that the correct volumes of ethanol (96-100%) is added into W1 and Wash Buffer when first open. Repeat the extraction procedure with a new sample.
The volume or the percentage of ethanol is not correct before adding into W1 and Wash Buffer	Make sure that the correct volumes of ethanol (96-100%) is added into W1 and Wash Buffer when first open. Repeat the extraction procedure with a new sample.

Possible reasons	Solutions
Elution of genomic DNA is not efficient	
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.
Elution Buffer or ddH_2O is not completely absorbed by column membrane	After Elution Buffer or ddH_2O is added, stand the FABG Maxi Column for 5 min before centrifugation.
Column is clogged	
Blood sample contains clots	Repeat the extraction procedure with a new sample. Mix the blood sample well with anti-coagulant to prevent formation of blood clots.
Sample is too viscous	Reduce the sample volume.
Degradation of elutated DNA	
Sample is old	Always use fresh or well-stored sample for genomic DNA extraction.
Buffer for gel electrophoresis contaminated with DNase	Use fresh running buffer for gel electrophoresis.