FISHER MOLECULAR BIOLOGY

Blood Genomic DNA Extraction Mini Kit

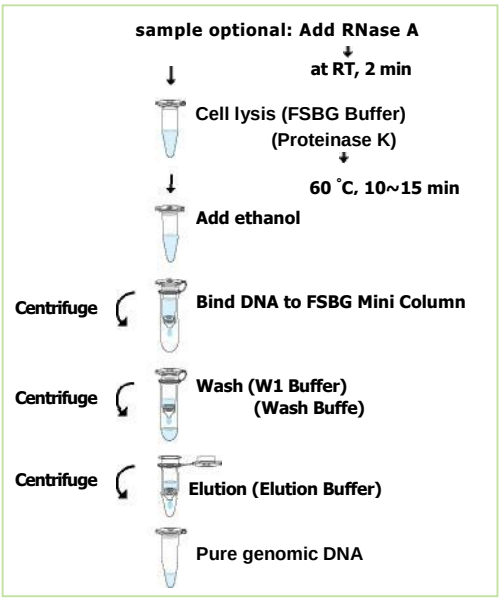
Kit Contents:

	DE-014s (4 preps_ sample)	DE-014 (50 preps)	DE-015 (100 preps)	DE-016 (300 preps)
FSBG Buffer	1.5 ml	15 ml	30 ml	70 ml
W1 Buffer * (concentrate)	1.3 ml	22 ml	44 ml	124 ml
Wash Buffer ** (concentrate)	1 ml	10 ml	20 ml	50 ml
Elution Buffer	1 ml	15 ml	30 ml	90 ml
Proteinase K	1 mg	11 mg	11 mg x 2	11 mg x 6
FSBG Mini Column	4 pcs	50 pcs	100 pcs	300 pcs
Collection Tube	8 pcs	100 pcs	200 pcs	600 pcs
Elution Tube	4 pcs	50 pcs	100 pcs	300 pcs
User Manual	1	1	1	1

* Preparation of W1 Buffer, Wash Buffer and proteinase K solution for first use:				
Cat. No:	DE-014S (4 preps)	DE-014 (50 preps)	DE-015 (100 preps)	DE-016 (300 preps)
Ethanol volume for W1Buffer *	0.5 ml	8 ml	16 ml	45 ml
Ethanol volume for Wash Buffer **	4 ml	40 ml	80 ml	200 ml
ddH2O volume for Proteinase K solution†	0.1 ml	1.1 ml	1.1 ml	1.1 ml

Specification:

Principle: spin column (silica membrane)
Sample: up to 200 µl whole blood, serum, plasma, body fluids up to 5 x 10^6 cultured cells
Operation time: < 30 min
Binding capacity: up to 60 µg/ column
DNA Yield: 4~8 µg/ 200 µl of whole blood



Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Store proteinase K tube at -20 °C. Before first use, add required volume of sterile ddH2O to Proteinase K tube to make a 10 mg/ml stock solution. Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4 °C.**
3. Add required volume of ethanol (96- 100 %) to W1 Buffer and Wash Buffer when first use.
4. Preheat a dry bath or water bath to 60 °C before the operation.
5. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.

General Protocol:

HINT: Prepare a dry bath or water bath to 60 °C bath for step 4. Preheat Elution Buffer to 65 °C for step 13 (elution step).

Please Read Important Notes Before Starting The Following Steps.

1. Transfer up to 200 µl sample (whole blood, serum, plasma, body fluids, buffy coat) to a microcentrifuge 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 min at room temperature.
3. Add 20 µl Proteinase K and 200 µl FSBG Buffer to the sample. **Mix thoroughly by pulse-vortexing.** - Do not add Proteinase K directly to FSBG 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 10 sec.**
7. Briefly spin the tube to remove drops from the inside of the lid.
8. Place a FSBG Mini Column to a Collection Tube. Transfer the mixture (including any precipitate) carefully to the FSBG Mini Column. Centrifuge at 6,000 x g for 1 min **then place FSBG Mini Column to a new Collection Tube.**
9. Add 400 µl W1 Buffer to the FSBG Mini Column and centrifuge at full speed (18,000 x g) for 30 sec then discard the flow-through.
10. Add 750 µl Wash Buffer to the FSBG Mini Column and centrifuge at full speed for 30 sec then discard the flow-through. - Make sure that ethanol has been added into Wash Buffer when first open.
11. **Centrifuge at full speed for an additional 3 minutes to dry the column.**
    Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.
12. Place the FSBG Mini Column to a Elution Tube.
13. Add 50 ~ 200 µl of Elution Buffer or ddH2O (pH 7.5- 9.0) to the membrane center of FSBG Mini Column. **Stand FSBG Mini Column for 3 minutes.**
    - **Important Step!** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
14. Centrifuge at full speed for 1 minutes to elute total DNA.
15. Store total DNA 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 1.5 ml microcentrifuge tube.
II. Centrifuge at 300 x g for 5 min.
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. 6
II. Transfer the appropriate number of cell (up to 5 x 10^6) to a 1.5 ml microcentrifuge tube.
III. Centrifuge at 300 x g for 5 min.
IV. Remove the supernatant carefully and completely.

2. Resuspend cell pellet in PBS to a final volume of 200 µl.
3. Follow the General Protocol starting from step 2.

Preparation of buffy coat

Centrifuge whole blood at 3,300 x g for 10 min 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.

Troubleshooting

Possible reasons	Solutions
Low or no yield of genomic DNA Low amount of cells in the sample	Concentrate a larger volume of a new sample to 200 ul. If the sample is whole blood, prepare buffy coat
Poor cell lysis	
Poor cell lysis because of insufficient Proteinase K activity	Repeat the extraction procedure with a new sample. Use a fresh or wellstored Proteinase K stock solution.
Poor cell lysis because of insufficient mixing with FSBG buffer	Repeat the extraction procedure with a new sample. Mix the sample and FSBG Buffer immediately and thoroughly by pulse-vortexing.
Poor cell lysisbecause 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 FSBG Mini Column	Repeat the extraction procedure with a new sample.
Incorrect preparation of Wash Buffer	
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.
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.
Elution of genomic DNA is not efficient	
pH of water (ddH2O) for elution is acidic	Make sure the pH of ddH2O is between 7.5- 9.0.
	Use Elution Buffer (provided) for elution.
Elution Buffer or ddH2O is not completely absorbed by column membrane	After Elution Buffer or ddH2O is added, stand the FSBG Mini 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.

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