ISOSPIN Blood & Plasma DNA

Manual (Ver. 02)

Code No. 312-08131

NIPPON GENE CO., LTD.

I Description

The ISOSPIN Blood & Plasma DNA enables a rapid isolation of highly pure DNA from blood, serum and plasma.

II Kit components

Componet	(50 preps)	Note
BE Buffer	15 ml x 1	
Proteinase K	1 ml x 1	
BW1 Buffer	48 ml x 1	(includes ethanol)*
BW2 Buffer	35 ml x 1	(includes ethanol)*
Elution Buffer	14 ml x 1	10 mM Tris-HCl (pH 9.0), 0.1 mM EDTA
Spin Column	50 sets x 1	(a Spin Column and Collection Tube set)
Collection Tube	50 tubes x 2	

* Keep the buffer bottles tightly closed after use.

III Storage conditions

Proteinase K should be stored at -20°C. The remaining kit components can be stored at room temperature (15-25°C).

IV Precautions

・ The kit is intended research use only.

V Protocol

Material not supplied

・Ethanol
・Micropipette
・Pipette tips
・1.5 mL microcentrifuge tubes
・Microcentrifuge
・Vortex Mixer
・Water baths or heat blocks (at 56°C)

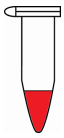
Material, as necessary

・Saline solution (Do not use buffer solution such as PBS.)
・Ribonuclease (DNase free) Glycerol Solution (Nippon Gene, Code No. 312-01931)

Before You Begin

☐ Warm blood samples to room temperature (15-25°C).
☐ Set a water baths or heat block at 56°C.

DNA Extraction Protocol



1. Add 20 µl of **Proteinase K** to a 1.5 mL microcentrifuge tube.

2. Warm **blood samples** to room temperature (15-25°C), and mix well by inversion. Add 200-250 µl of the sample to the tube.
   Note) If using <200 µl blood sample, adjust the sample volume to 200 µl using saline solution. (Do not use buffer solution such as PBS. Using PBS may cause inefficient DNA extraction.)
   Note) If using nucleated red blood (*e.g.*, bird, reptile, amphibian or fish), add 190 µl of saline solution to 10 µl of nucleated red blood (the sample will be 200 µl as a starting material.)

Opt. *OPTIONAL STEP (A)*

a1) If RNase treatment is required, add 20 µl of Ribonuclease (DNase free) Glycerol Solution to the sample, and mix. Spin down lightly.

3. Add 1 sample volume (200-250 µl) of **BE Buffer** to the tube, mix well by vortexing for 15 sec. Spin down lightly.

4. Incubate at 56°C for 10 min.

5. Add 1 sample volume (200-250 µl) of **96-100% Ethanol** to the tube, mix well by vortexing for 15 sec. Spin down lightly.

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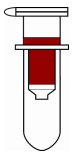

6. Prepare a **Spin Column** (a Spin Column and Collection Tube set). Apply all the **mixture** to the Spin Column.
   Note) If using >200 µl serum or plasma sample, you can apply any remaining mixture to the same Spin Column several times (up to 5 times) by repeating Step 6 and Step 7. But, to avoid clogging is preferable to further addition.

7. Centrifuge at 12,000 x g, for 1min at room temperature.

8. Discard the flow-through with the Collection Tube. Place the Spin Column into a clean **Collection Tube**.

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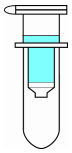

9. Add 750 µl of **BW1 Buffer** to the Spin Column.

10. Centrifuge at 12,000 x g, for 1 min at room temperature.

11. Discard the flow-through with the Collection Tube. Place the Spin Column into a clean **Collection Tube**.

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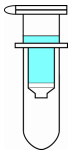

12. Add 500 µl of **BW2 Buffer** to the Spin Column.

13. Centrifuge at 12,000 x g, for 5 min at room temperature.

14. Discard the flow-through with the Collection Tube. Transfer the Spin Column to a new 1.5 mL microcentrifuge tube.
Note) Avoid contact of the Spin Column with the flow-through. If in doubt, perform next Optional Step (B).

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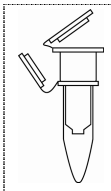

Opt. *OPTIONAL STEP (B)*

b1) Centrifuge at 12,000 x g, for 1 min at room temperature to remove residual liquid.

b2) Discard the flow-through with the 1.5 mL microcentrifuge tube. Transfer the Spin Column to a new 1.5 mL microcentrifuge tube.

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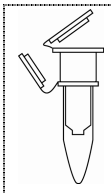


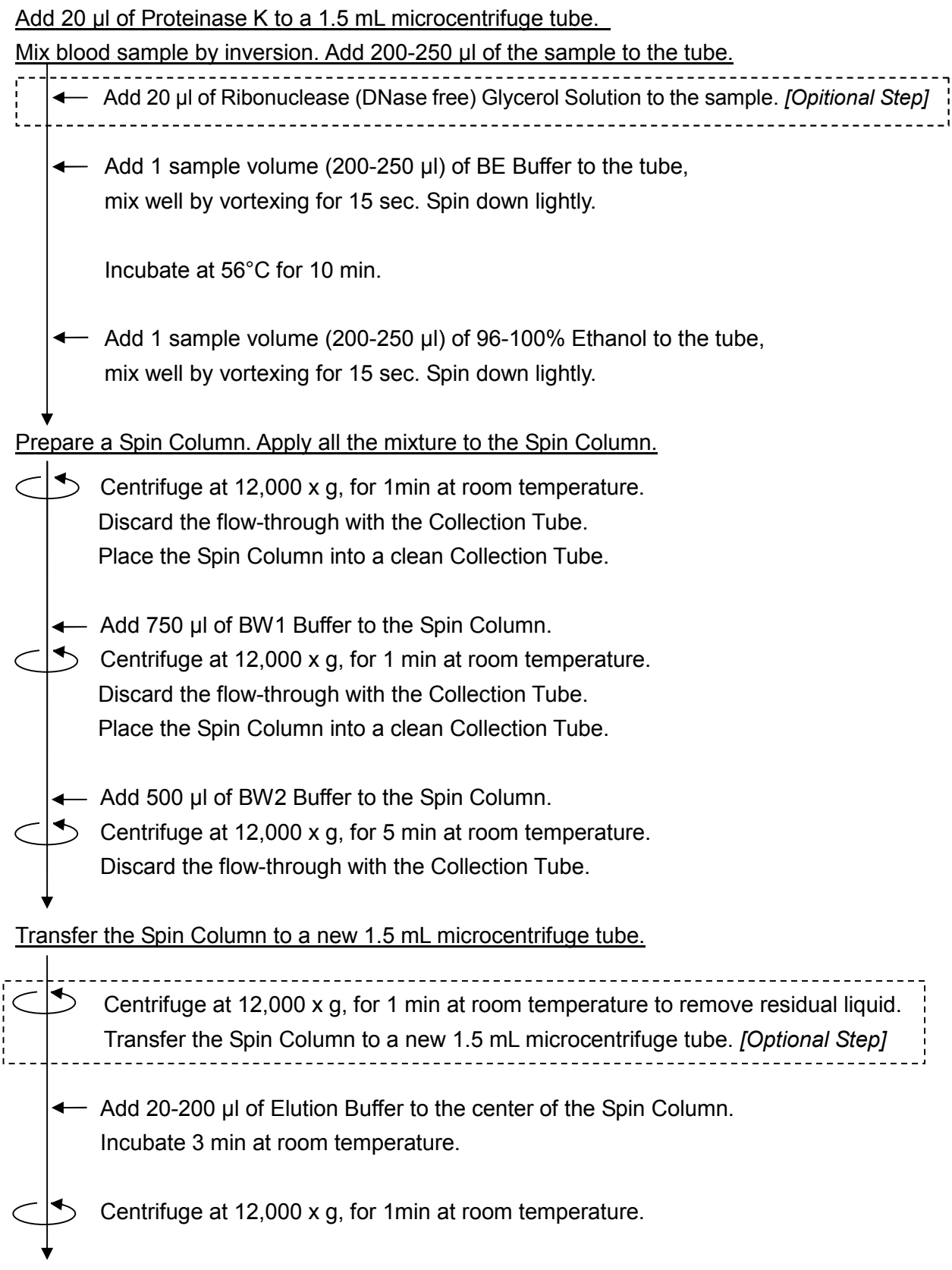
15. Add 20-200 µl of **Elution Buffer** to the center of the Spin Column. Incubate 3 min at room temperature.
Note) Nuclease-free water can also be used to elute the DNA in place of Elution Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 9.0). We recommend using Elution Buffer containing EDTA if you store the eluted DNA for long-term storage.
Note) Larger elution volumes can increase yield of DNA at the cost of dilution of the sample (see the page 7.)

16. Centrifuge at 12,000 x g, for 1min at room temperature.

17. Recover your purified DNA in the microcentrifuge tube.
Note) The purified DNA can be used directly or stored at -20°C for long term storage.

Simplified Protocol

Warm blood (serum or plasma) samples to room temperature (15-25°C).



VI Troubleshooting

Low DNA yield

When adding BE Buffer, mix well by vortexing for more than 15 sec.
When adding 96-100% Ethanol, mix well by vortexing for 15 sec.
For increased DNA yield, add 100-200 µl of Elution Buffer in Step 15.
Do not use distilled water less than or equal to pH7.0, instead of Elution Buffer in Step 15.

Discolored membrane after washing with BW1 Buffer

When adding BE Buffer, mix well by vortexing for more than 15 sec.
Wash again with BW1 Buffer (repeat the Steps 8-11, before the Step 12.)

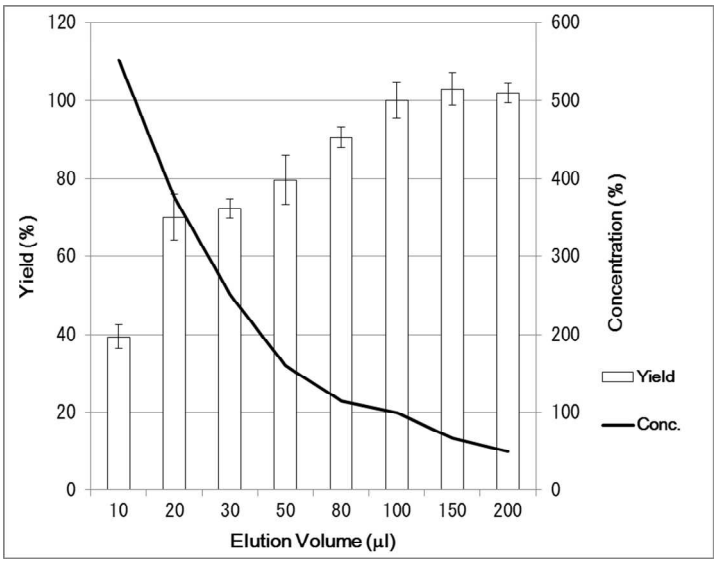
Low concentration of DNA

For higher concentration, add 20-50 µl of Elution Buffer in Step 15. Smaller elution volumes can increase concentration of DNA at the cost of yield of the sample.

Low DNA Performance

Perform the Optional Step (B) to remove BW2 Buffer from the Spin Column.

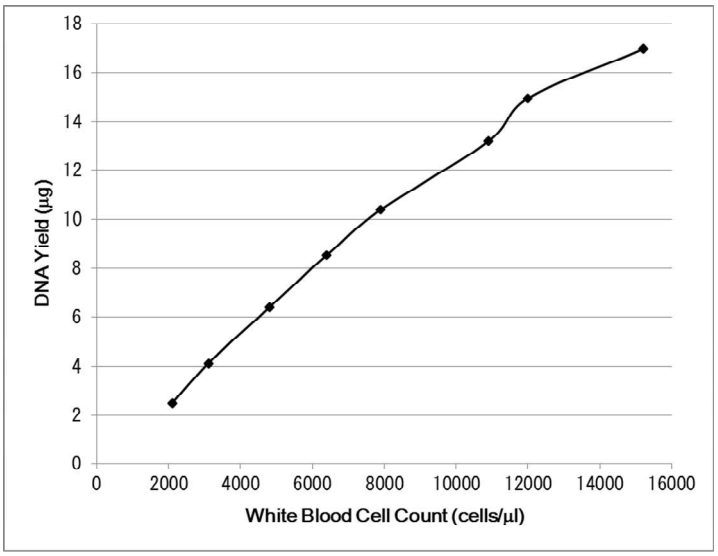
VII Data



The DNA yield and concentration varies with Elution Buffer volume.

Yield and concentration using different elution volumes are expressed as a percentage of the sample using a 100 µl elution volume.

For each Elution volume, the relative yield (bar chart) and the relative concentration (line chart) are depicted in this data.



The DNA yield varies with white blood cell count.

Genomic DNA was purified from 100 µl human whole blood with the kit using a 100 µl Elution Buffer.

VIII Rerated products

Code No.	Product Name	Size
312-01931	Ribonuclease (DNase free) Glycerol Solution	1 ml
319-08141	Collection Tube	100 tubes
315-08143		500 tubes

NIPPON GENE CO., LTD.

URL: http://www.nippongene.com/

If you have any questions, please contact us by web form.