Genomic DNA Extraction from whole blood samples using upgraded Exgene™ Blood SV mini

Experimental Conditions

Materials Required

- [105-101] Exgene™ Blood SV mini (old; O)
- [105-101] Exgene™ Blood SV mini (new; N)
- DNA extraction commercial kit for blood (supplier A)

Sample & Extraction Information

Sample type	Whole blood
Origin	Human
Target	Genomic DNA
Sample amount	200 µl
Elution volume	200 µl

Improvement Information

Components	Improvement points
Buffer BL → Buffer JBL	Lysis efficiency
Buffer BW → Buffer JBW	Minimum precipitation
Buffer AE → Nuclease-free Water	Removal of PCR inhibitor

Protocol

For more details and methods, please refer to the manual of Exgene™ Blood SV mini.

Preparation of Proteinase K Solution

To obtain 20 mg/ml of proteinase K solution, add 2.4 ml of PK Storage Buffer to one bottle of lyophilized Proteinase K (48 mg), and gently invert to dissolve.

Sample Preparation

1. Add 20 µl of proteinase K solution (20 mg/ml, provided) into the bottom of a 1.5 ml microcentrifuge tube (not provided).
2. Transfer 200 µl of sample to the tube.
3. Add 200 µl of Buffer JBL to the tube. Vortex the tube to mix thoroughly. Incubate at 56 °C for 10 min. Spin down briefly to remove any drops from inside of the lid.
4. Add 200 µl of chilled absolute ethanol (not provided) to the sample, vortex 30 s to mix the sample thoroughly and spin down briefly to remove any drops from inside of the lid.
5. Transfer the mixture to the Column Type G (mini) carefully, centrifuge at 13,000 rpm for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.
6. Add 500 µl of Buffer JBW, centrifuge at 13,000 rpm for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube (1st washing).
7. Repeat step 6 (2nd washing).
8. Apply 700 µl of Buffer NW. Centrifuge at 13,000 rpm for 1 min (3rd washing). Replace the collection tube with new one (provided).
9. Centrifuge at full speed for 3 min to remove residual wash buffer. Place the mini column in a fresh 1.5 ml micro centrifuge tube (not provided).
10. Add 200 µl of Nuclease-free Water to the membrane. Incubate for 2 min at room temperature.
11. Centrifuge at full speed for 2 min at room temperature.

* Each standard protocol was adopted for Exgene™ Blood SV mini (O) and supplier A's commercial kit.

Result

Kit	Yield (µg)		A_{260}/A_{280}		A_{260}/A_{230}	
	Mean	CV	Mean	CV	Mean	CV
Exgene™ Blood SV mini (O)	0.8	0.41	1.42	0.12	0.38	0.78
Exgene™ Blood SV mini (N)	6.0	0.22	1.74	0.13	1.18	0.61
Supplier A	2.5	0.10	1.78	0.05	1.18	0.27

Table 1. DNA yield and purity
DNA was extracted in duplicate from human whole blood using the upgraded Exgene™ Blood SV mini (N) and two comparative kits (O, supplier A). The upgraded kit demonstrated superior yield and purity, confirmed by NanoDrop™ 2000 analysis.

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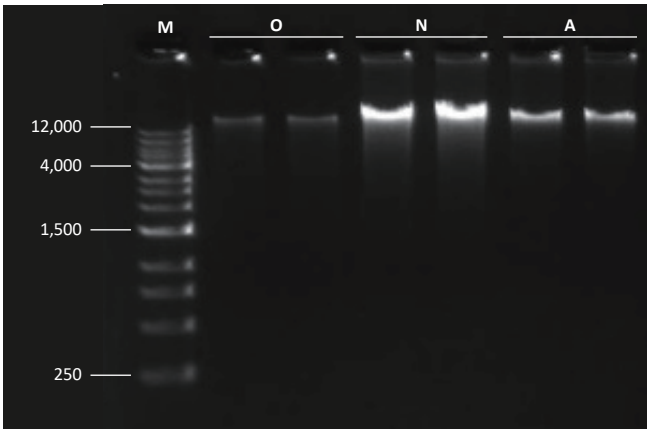
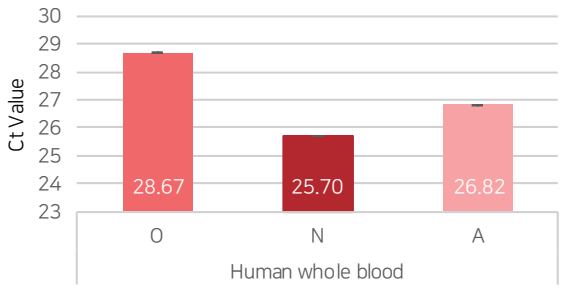


Figure 1. DNA integrity
DNA extracts were visualized on a 1.5 % agarose gel.
Electrophoresis was performed at 100 V for 25 minutes using 0.5 X TBE Buffer and the GENESTA™ 1 kb DNA Ladder (GA-100) as a reference.

O: Exgene™ Blood SV mini (old)
N: Exgene™ Blood SV mini (new)
A: DNA extraction commercial kit for blood (supplier A)



Sample ID	Ct Value		
	O	N	A
Blood 1	28.92	25.57	26.65
Blood 2	28.41	25.83	26.98
Mean	**28.67**	**25.70**	**26.82**
CV	**0.26**	**0.13**	**0.17**

Figure 2. Real-time PCR
qRT PCR data for human GAPDH gene (79 bp) were amplified from DNA extracts. qPCR was performed with HyperScript™ One-step RT-PCR Master Mix (602-110) on the CFX96™ System (1855201).

O: Exgene™ Blood SV mini (old)
N: Exgene™ Blood SV mini (new)
A: DNA extraction commercial kit for blood (supplier A)

Summary

- The enhanced version of the Exgene™ Blood SV mini demonstrates higher performance compared to both the previous version and a competitor's kit.
- The improved buffer components enhanced DNA yield and purity, resolved precipitation issues in the washing buffer, for user convenience.

Ordering Information

Cat. No.	Product	Size
105-101	Exgene™ Blood SV mini	100 preps
105-152	Exgene™ Blood SV mini	250 preps