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			
Origin			
Target	Genomic DNA		
Sample amount	ample amount 200 μl		
Elution volume	200 μΙ		

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 μ l 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 μ l 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 μ l 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 μ l 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 μ l 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 μ l 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.

Result

Kit	Yield (μg)		A ₂₆₀ /A ₂₈₀		A ₂₆₀ /A ₂₃₀	
KIL	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.

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

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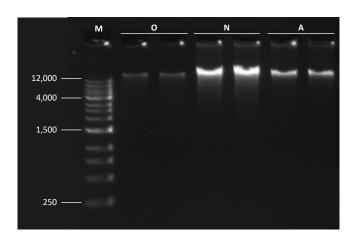


Figure 1. DNA integrity

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 $^{\rm m}$ 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)

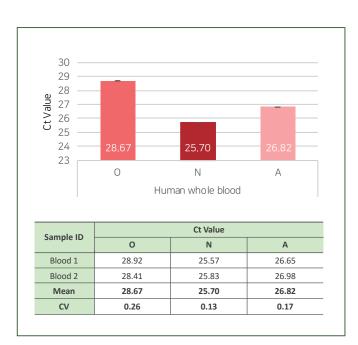


Figure 2. Real-time PCR

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