GeneAll[®] Application Note

Genomic DNA extraction with GenEx[™] Blood from human whole blood and buffy coat (PBMC) isolated from human whole blood

Experimental Conditions

Materials Required

- GenEx[™] Blood Sx (100 prep : 220-101 / 500 prep : 220-105)
- Isopropanol (C₃H₈O, CAS : 67-63-0)
- 70% ethanol (C₂H₅OH, CAS : 64-17-5)
- 15 ml centrifuge tube
- EDTA vacuum tube (for human whole blood)
- Microcentrifuge (≤14,000 x g)
- Vortex mixer
- Pipette & sterilized pipette tips
- Suitable protector (e.g., lab coat, disposable gloves, goggles, etc.)

Sample Information

Extraction conditions

Sample	Amount	Elutoin volume
Human whole blood	3 ml	250 μl
Buffy coat (PBMC)	150 μl	

Protocol

GenEx[™] Blood Sx Protocol

* For more details and methods, please refer to the handbook of GenEx[™] Blood/ Cell/Tissue.

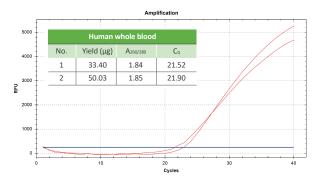
Sample Preparation

- Human whole blood
- 1. Transfer 9 ml of Buffer RL to a fresh 15 ml centrifuge tube.
- Add 3 ml of whole blood to the tube containing Buffer RL. Invert the tube 5~6 times to mix. Incubate the mixture for 10 min at room temperature.
- 3. The subsequent protocol follows <u>step 3 on page 18 of B.</u> <u>Protocol for 3 ml Whole Blood in the GenEx[™] Blood/Cell/</u> <u>Tissue handbook.</u>

• Buffy coat (PBMC)

- 1. Centrifuge the 3 ml of human whole blood in EDTA vacuum tube at $2,000 \times \text{g}$ above for 10 min at $15 \sim 25^{\circ}\text{C}$.
- 2. After separating the plasma layer, carefully separate the intermediate buffy coat to a new tube and transfer the 150 μ l of buffy coat to 15 ml centrifuge tube.
- 3. Add the 450 μl of Buffer RL to 15 ml centrifuge tube and mix by inverting 5~6 times.
- Incubate the mixture for 10 min at room temperature. Invert 4~5 times during the incubation.
- 5. The subsequent protocol follows <u>step 3 on page 18 of B.</u> <u>Protocol for 3 ml Whole Blood in the GenEx[™] Blood/Cell/</u> <u>Tissue handbook.</u>

Result



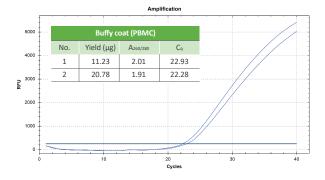


Figure 1. Genomic DNA extraction and quality assessment from human blood and buffy coat.

Genomic DNA was extracted from human whole blood and buffy coat using GenEx[™] Blood Sx (220-101) in duplicate. The quantification of DNA was carried out using NanoDrop[™] 2000 (ND-2000, Supplier : T) spectrophotometer. To assess the quality of the extracted DNA, real-time PCR was performed using Human GAPDH primers with the RealAmp[™] 2X qPCR Master Mix (801-020) on the CFX96[™] System (1855201, Supplier : B).