

Application of Haier LX-155T500R and LX-60T750R Refrigerated Centrifuges for PBMC Isolation from Peripheral Blood

Abstract

Efficient isolation of peripheral blood mononuclear cells (PBMCs) is a critical prerequisite for immunology research, cell therapy, and clinical diagnostics. This study evaluated the performance of two Haier Biomedical refrigerated centrifuges, the LX-155T500R and LX-60T750R, for PBMC isolation using Ficoll-Paque™ density gradient centrifugation. Centrifugation parameters were set at 400×g, 30 minutes, 20°C, acceleration level 9, and deceleration level 0 (natural stop). Both centrifuges produced a clear four-layer separation structure. The LX-155T500R yielded approximately 2.61×10^6 PBMCs per mL of whole blood with 99.74% cell viability, and the LX-60T750R yielded approximately 2.075×10^6 PBMCs per mL with 99.84% viability. Both results fall within the commonly reported range in the literature ($1.5\text{--}3.0 \times 10^6$ cells/mL), confirming expected separation performance. A blood freshness experiment further demonstrated that samples collected within 2 hours yielded the best separation quality, while samples stored beyond 72 hours showed significant degradation. In conclusion, both centrifuges achieve efficient, high-viability PBMC isolation and meet the requirements of research and clinical applications.

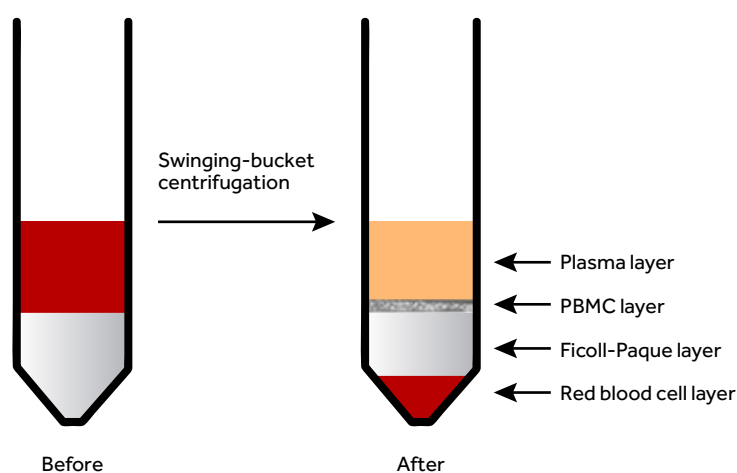
Keywords: peripheral blood mononuclear cells (PBMC); density gradient centrifugation; Ficoll-Paque™; Haier centrifuge; LX-155T500R; LX-60T750R

1. Introduction

Peripheral blood mononuclear cells (PBMCs) primarily consist of lymphocytes and monocytes. They are widely used in immunology research, cell therapy, vaccine development, and clinical diagnostics. Efficient isolation of high-viability PBMCs from whole blood is a key prerequisite for downstream experimental success.

Density gradient centrifugation is the classical method for PBMC isolation, originally established by Bøyum in 1968^[1]. The technique exploits density differences among blood cell types. During centrifugation, cells distribute across interfaces of media with different densities. Ficoll-Paque™ separation medium (density 1.077 g/mL) positions PBMCs at the plasma-medium interface, while erythrocytes and granulocytes pellet at the tube bottom^[2]. Acceleration and deceleration control during centrifugation is critical for interface clarity. Excessive deceleration can remix already-separated layers and compromise isolation quality.

Haier Biomedical has developed the high-speed LX-155T500R and the low-speed LX-60T750R refrigerated centrifuges with precise speed control, temperature regulation, and multi-level acceleration/deceleration settings. This study systematically evaluates the performance of both models for PBMC isolation, providing laboratory users with a standardized operating protocol.



2. Materials and Methods

• 2.1 Materials

Material / Equipment	Specification / Model
High-speed refrigerated centrifuge	Haier LX-155T500R (with swing-bucket rotor 4×500 mL and 15 mL tube adapters)
Low-speed refrigerated centrifuge	Haier LX-60T750R (with swing-bucket rotor 4×750 mL)
Biological safety cabinet	Haier HR1500-IIA-DW
Anticoagulated whole blood	Freshly collected, EDTA anticoagulant
PBMC separation medium	Cytiva, Ficoll-Paque™ PLUS, density 1.077 g/mL at 20 °C
PBS buffer	pH 7.2–7.4
Centrifuge tubes	15 mL conical-bottom tubes
Cell counter	Countstar Mira FL
Fluorescent cell dye	AO/PI, Countstar

• 2.2 Sample Preparation

1. Equilibrate Ficoll-Paque™ medium and PBS buffer to room temperature (18–25°C).
2. Mix the Ficoll-Paque™ medium by gentle inversion.
3. Dilute fresh anticoagulated whole blood with PBS at a 1:1 volume ratio. Mix gently by inversion.
4. Add 3 mL of Ficoll-Paque™ medium to a 15 mL conical tube.
5. Carefully layer 4 mL of diluted blood on top of the Ficoll-Paque™ medium. Avoid disturbing the interface.
6. Prepare duplicate samples following the steps above for separate centrifugation on each instrument.

• 2.3 Centrifugation Parameters

Parameter	LX-155T500R	LX-60T750R
Centrifugal force	400×g	400×g
Duration	30 min	30 min
Temperature	20°C	20°C
Acceleration	Level 9	Level 9
Deceleration	Level 0 (natural stop)	Level 0 (natural stop)
Rotor type	Swing-bucket rotor 4×500 mL	Swing-bucket rotor 4×750 mL

• 2.4 PBMC Harvesting and Washing

1. After centrifugation, carefully remove the tube without disturbing the separated layers.
2. Use a 1 mL pipette to remove the top plasma layer.
3. Collect the PBMC layer (second layer, approximately 1 mL) and transfer to a clean tube.
4. Add 4 volumes of PBS buffer, mix gently. Total volume is approximately 5 mL.
5. Centrifuge at 220×g for 10 min (4°C, acceleration level 9, deceleration level 9) to pellet the PBMCs.
6. Repeat the PBS wash 1–2 times.
7. Collect the PBMC pellet for cell counting and viability assessment.
8. Resuspend the pellet in 1 mL PBS. Mix 20 µL of cell suspension with 20 µL AO/PI dye. Load 20 µL onto a counting chamber and record cell concentration and viability using an automated cell counter.

3. Results

Note: The separation experiments for the LX-155T500R and LX-60T750R were performed independently using blood samples from different donors. Variations in cell yield between the two instruments are expected due to biological differences among donors (e.g., baseline leukocyte count, blood viscosity). Both yields fall within the literature-reported standard range, confirming that separation performance meets expectations for both models.

● 3.1 Separation Performance of LX-155T500R

Centrifugation of the LX-155T500R at 400×g for 30 minutes at 20°C with deceleration set to level 0 (natural stop) produced a clearly defined four-layer structure (Figure 1):

- Layer 1: Light yellow plasma layer.
- Layer 2: Dense white PBMC layer (lymphocytes and monocytes).
- Layer 3: Transparent Ficoll-Paque™ medium layer.
- Layer 4: Erythrocyte and granulocyte pellet at the bottom.

The four layers were clearly delineated with a compact and well-defined PBMC band that was easy to identify and collect. Cell counting revealed a total of 5.22×10^6 PBMCs recovered from the whole blood sample, corresponding to approximately 2.61×10^6 cells per mL of whole blood, with a cell viability of 99.74% (Figure 2).

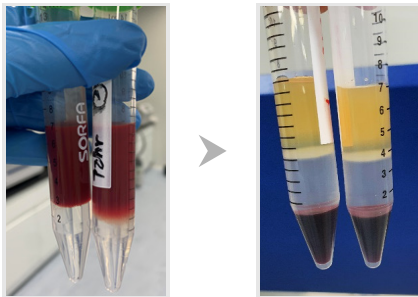


Figure 1. PBMC separation by LX-155T500R showing the four distinct layers.

Figure 2. Cell count and viability of washed PBMC pellet (99.74% viability).

● 3.2 Separation Performance of LX-60T750R

The LX-60T750R achieved equally effective PBMC separation under identical centrifugation parameters (400×g, 30 min, 20°C, acceleration level 9, deceleration level 0) (Figure 3). The layered structure was clear, with the PBMC band accurately positioned at the expected interface.

Cell counting showed a total of 4.15×10^6 PBMCs recovered, corresponding to approximately 2.075×10^6 cells per mL of whole blood, with a cell viability of 99.84% (Figure 4).

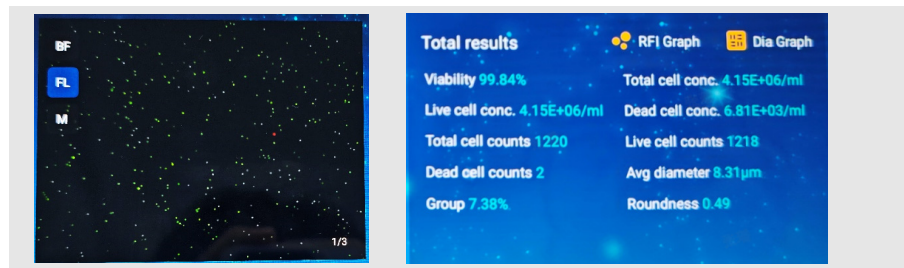
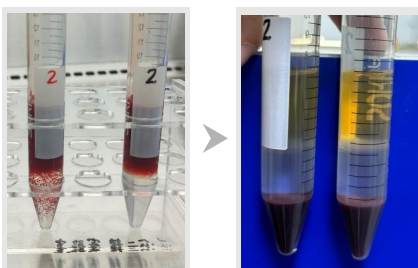


Figure 3. PBMC separation by LX-60T750R.

Figure 4. Cell count and viability of washed PBMC pellet (99.84% viability).

● 3.3 Effect of Blood Freshness on Separation Quality

To assess the impact of sample freshness on PBMC isolation, anticoagulated whole blood was stored at 4°C and processed at 72 hours and 148 hours post-collection. Results are summarized below (Figure 5):

Storage Time	Layer Quality	Cell Status
<2 hr (fresh)	Clear layers, sharp interfaces	High viability, culturable
72 hr	Blurred interface, irregular granules	Reduced viability
148 hr	Significantly degraded, hemolysis in plasma	Fragmented non-culturable

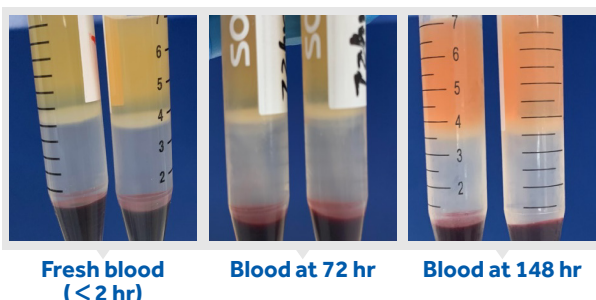


Figure 5. Effect of blood storage duration on PBMC layer quality (left: fresh blood <2 hr, clear separation; center: 72 hr, blurred interface; right: 148 hr, significantly degraded separation).

4. Discussion

This study systematically evaluated the PBMC isolation performance of the Haier LX-155T500R and LX-60T750R refrigerated centrifuges. Both instruments achieved efficient, high-viability PBMC separation. Cell yields and viability levels met the requirements for research and clinical applications.

● Key Findings on Centrifugation Parameters

The centrifugation protocol used in this study (400×g, 30 min, 20°C, acceleration level 9, deceleration level 0) is fully consistent with the parameters recommended in the Cytiva Ficoll-Paque™ PLUS product data sheet [2]. Deceleration control proved to be particularly important. Rapid deceleration can remix the separated layers and compromise interface clarity. Level 0 deceleration (natural stop) allows the centrifugal force to decrease gradually from 400×g to zero, effectively preventing layer disturbance and preserving separation quality.

● Impact of Blood Freshness

Sample freshness had a significant impact on PBMC isolation quality. Fresh blood (collected within 2 hours) produced the best results, with clear layer separation and high cell viability. Samples stored for more than 72 hours showed markedly reduced separation quality and lower cell viability, making them unsuitable for downstream applications. These findings highlight the importance of using freshly collected blood for PBMC isolation to obtain high-quality cell samples.

● Instrument Performance

Both the LX-155T500R and LX-60T750R are equipped with variable-frequency motors and precision control systems that enable smooth acceleration and deceleration profiles. The swing-bucket rotor design keeps tubes in a horizontal orientation during centrifugation, which facilitates the formation of well-defined density gradient interfaces. Temperature control accuracy of ±1°C ensures thermal stability throughout the run, supporting optimal cell viability.

● Comparison with Published Data

Patrone et al. [3] reported PBMC yields of approximately $1.5\text{--}3.0 \times 10^6$ cells per mL of whole blood. The yields obtained in this study (2.075×10^6 and 2.61×10^6 cells/mL) are consistent with or exceed this range, indicating that the separation performance of both Haier centrifuge models is competitive within the industry.

5. Conclusion

The Haier high-speed LX-155T500R and low-speed LX-60T750R refrigerated centrifuges demonstrated excellent performance for PBMC isolation:

- Excellent layer separation: A clear four-layer structure was obtained under the conditions of 400×g, 30 min, 20°C, acceleration level 9, and deceleration level 0.
- High cell yield: Over 2.0×10^6 PBMCs per mL of whole blood were recovered, consistent with literature-reported ranges.
- Superior cell viability: PBMC viability exceeded 99% for both instruments.
- Practical recommendation: Use blood samples collected within 2 hours for optimal separation results.

In summary, the Haier LX-155T500R and LX-60T750R refrigerated centrifuges are reliable tools for PBMC isolation, capable of delivering high-quality cell samples for immunology research, cell therapy, and clinical diagnostics.

References

- [1] Bøyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scandinavian Journal of Clinical and Laboratory Investigation. 1968;21(Suppl 97):77-89.
- [2] Cytiva. Ficoll-Paque™ PLUS: Density gradient media for isolation of cells and viruses — Product Data Sheet. Cytiva Life Sciences, 2023. <https://cdn.cytivalifesciences.com/api/public/content/digi-15478-pdf>
- [3] Patrone D, Alessio N, Antonucci N, Brigida AL, Peluso G, Galderisi U, Siniscalco D. Optimization of Peripheral Blood Mononuclear Cell Extraction from Small Volume of Blood Samples: Potential Implications for Children-Related Diseases. Methods and Protocols. 2022;5(2):20. doi: 10.3390/mps5020020.

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