# ESTABLISHMENT OF A LYMPHOBLAST CULTURE PROTOCOL FROM PERIPHERAL BLOOD

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## ABSTRACT

Lymphoblasts, as lymphocyte precursors, are vital for research in cellular biology, immunology, genetics, and therapeutic development due to their proliferative potential. This study introduces a standardized protocol for isolating, transforming, and culturing lymphoblasts from peripheral blood of three healthy volunteers (≥18 years). The method uses Ficoll-Hypaque gradient centrifugation to isolate mononuclear cells, followed by Epstein-Barr virus (EBV) transformation and culture in RPMI-1640 medium with fetal bovine serum (FBS), phytohemagglutinin (PHA), and antibiotics at 37°C with 5% $CO_2$. Growth was assessed via Giemsa staining, automated cell counting, and Trypan Blue viability assays. The protocol achieved high lymphocyte purity (up to 90%), a mean 37-fold proliferation over 12 days, and 90.5% viability, ensuring reproducibility across samples. This standardized approach enables reproducible high-density lymphoblast cultures from minimal blood volumes, offering a robust platform for advancing studies in immunology, genetics, metabolic disorders, and cancer vaccine development. These results support progress in biotechnology research and therapeutic innovation.

***Keywords:*** *Lymphoblast culture, peripheral blood, cell isolation, Epstein-Barr virus, immunology, genetics*

## I. INTRODUCTION

Lymphoblastoid cell lines, generated by transforming peripheral blood lymphocytes with Epstein-Barr virus (EBV), are indispensable tools in immunology, genetics, and the study of metabolic disorders.[1-3] Their ability to proliferate indefinitely while preserving donor-specific characteristics makes them ideal for exploring immune responses, genetic variations, and metabolic pathways, including beta-oxidation disorders.[4,5] These cells also hold promise for advancing therapeutic innovations, such as cancer vaccines, by providing a stable platform for disease modeling and drug development.[6,7] However, establishing reliable lymphoblast cultures requires overcoming challenges related to standardized protocols, genetic stability, and contamination control.[8]

The development of such cultures is critical for expanding research capabilities, yet the process demands precise methodologies to ensure reproducibility and applicability. This study aims to establish a standardized protocol for the isolation, transformation, and culture of lymphoblasts using established techniques, including Ficoll-Hypaque centrifugation and EBV-induced transformation.[9] By optimizing these procedures, we seek to create a robust framework that supports future investigations in immunology, genetics, metabolic disorders, and cancer vaccine development.[10,11] This protocol will enhance the scientific foundation for studying complex diseases and therapeutic strategies, contributing to the advancement of biotechnology research. The focus on a reproducible and reliable methodology addresses the need for a consistent cell source, paving the way for broader applications in both basic and applied sciences.[1,11]

## II. MATERIALS AND METHODS

### 2.1. Study population and sample collection

Peripheral blood samples were obtained from three healthy adult volunteers (≥18 years). Each sample was collected in tubes containing Acid Citrate Dextrose (ACD) anticoagulant. *Inclusion criteria included:* complete participant information, appropriate blood volume (6 ml) in ACD tubes, processing within 6 hours, and normal complete blood count results. *Exclusion criteria comprised:* incomplete data, insufficient or poor-quality samples, processing beyond 6 hours, participants under immunosuppressive therapy, or individuals with hematologic malignancies.

### 2.2. Study design

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This was a cross-sectional descriptive study conducted at the Chemedic Vietnam Laboratory Center between December 2024 and May 2025.

### 2.3. Equipment and Reagents

The major equipment used is summarized in **Table 1**, and the reagents are listed in **Table 2**.

**Table 1.** Equipment used in the study

| No. | Equipment | Manufacturer/Origin |
|---|---|---|
| 1 | Centrifuge (EBA 20) | Hettich, Germany |
| 2 | Hematology Analyzer (D7-CRP) | Dymind, China |
| 3 | $CO_2$ Incubator | Memmert, Germany |
| 4 | Microscope | Carl Zeiss, Germany |
| 5 | Semi-automatic pipettes | Cleaver Scientific, UK |
| 6 | Laboratory consumables (tips, tubes, flasks) | Supplied with equipment |

**Table 2.** Reagents used in the study

| No. | Reagent | Supplier | Concentration/Use |
|---|---|---|---|
| 1 | Phosphate-buffered saline (PBS) | Servicebio, USA | 1×; blood dilution |
| 2 | EBSS | Servicebio, USA | 1×; PBMC washing |
| 3 | Histopaque®-1077 | Sigma, USA | Density gradient separation |
| 4 | RPMI-1640 medium | Servicebio, USA | Base culture medium |
| 5 | Fetal bovine serum (FBS) | Diagnovum, Germany | 20%; medium supplement |
| 6 | Epstein-Barr virus (EBV) | Kerafast, USA | Lymphocyte transformation |
| 7 | Penicillin/Streptomycin | Diagnovum, Germany | 100×; contamination prevention |
| 8 | Phytohemagglutinin (PHA) | Diagnovum, Germany | 20 µg/mL; lymphocyte stimulation |
| 9 | Absolute ethanol | Merck, Germany | 99.5%; fixation |
| 10 | Giemsa solution | Merck, Germany | 100% stock; 10% working solution |
| 11 | Trypan blue | Sigma, USA | 0.4%; viability assay |

### 2.4. Isolation and culture of lymphoblasts

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque (Histopaque-1077) density gradient centrifugation, as previously described.[12] Briefly, anticoagulated whole blood was diluted with PBS, layered over Histopaque, and centrifuged at 2000 rpm for 30 minutes. The mononuclear cell layer was collected, washed twice with EBSS, and resuspended in RPMI-1640 medium.

For lymphoblast transformation, PBMCs were cultured in RPMI-1640 supplemented with 20% heat-inactivated FBS, EBV, PHA, and penicillin/streptomycin. Cultures were maintained at 37°C in 5% $CO_2$, with medium renewal every 2–3 days by replacing 80% of the medium with fresh medium. Cells were cultured for 12 days, following established protocols for EBV-induced lymphoblastoid cell lines.[9]

### 2.5. Growth and viability assessment

*Morphology:* Cell morphology and size were evaluated by Giemsa staining according to standard cytological protocols.[13] Dried smears were fixed with absolute ethanol, stained with Giemsa (100% for 1 min, 10% for 10 min), and examined under a light microscope at 100× magnification.

*Cell count:* Cell concentration was determined using a Dymind D7-CRP automated hematology analyzer.

*Viability:* Viability was assessed using the Trypan Blue exclusion method, as previously described.[14] Equal volumes of cell suspension and Trypan Blue (0.4%) were mixed and counted within 5 minutes using a hemocytometer. Viability was calculated as:

$$\text{Viability (\%)} = \frac{\text{Number of viable cells}}{\text{Total number of cells}} \times 100$$

### 2.6. Cell storage

Cells in the logarithmic growth phase (~5 × $10^9$ cells/L) were cryopreserved in freezing medium (RPMI-1640 supplemented with 30% FBS and 5% DMSO). Aliquots were stored at –80°C overnight and subsequently transferred to liquid nitrogen (–196°C) for long-term storage.[6]

### 2.7 Ethical considerations

This study complied with the ethical principles of biomedical research. All participants were healthy volunteers who provided written informed consent after being informed of the

study objectives. Personal data were kept confidential. All procedures were performed under sterile conditions to ensure biosafety.

## III. RESULTS

### 3.1. Cell separation results from peripheral blood

**Table 3.1.** White blood cell and lymphocyte counts before and after separation

| Sample | Pre-separation | | | Post-separation | | |
|---|---|---|---|---|---|---|
| | WBC ($10^9$/L) | Lymph ($10^9$/L) | Lymph % | WBC ($10^9$/L) | Lymph ($10^9$/L) | Lymph % |
| MS.01 | 7.95 | 3.22 | 40.5 | 1.10 | 0.99 | 90.0 |
| MS.02 | 5.67 | 1.97 | 34.7 | 1.02 | 0.90 | 88.2 |
| MS.03 | 4.90 | 1.82 | 37.1 | 0.87 | 0.78 | 89.7 |
| Average | 6.17 | 2.34 | 37.43 | 1.00 | 0.89 | 89.0 |

**Table 1** summarizes white blood cell (WBC) and lymphocyte counts before and after Histopaque-Ficoll (density 1.077) separation. Pre-separation WBC counts ranged from 4.90 to 7.95 × $10^9$/L, with lymphocytes at 1.82–3.22 × $10^9$/L (34.7–40.5%). Post-separation, the average lymphocyte count was 0.89 × $10^9$/L, achieving 89.3% purity, with MS.01 yielding the highest recovery (0.99 × $10^9$/L, 90.0%).

### 3.2. Cell morphology and size

In suspension culture, lymphoblasts formed clusters or floated individually, showing oval-to-round morphology without adhesion. Giemsa staining (**Figure 1**) revealed cells of 10–20 µm, with nuclei occupying 80–90% of volume (dark purple) and thin, light blue cytoplasm. Over 90% exhibited normal morphology, with mitotic features in larger cells, indicating healthy cultures.

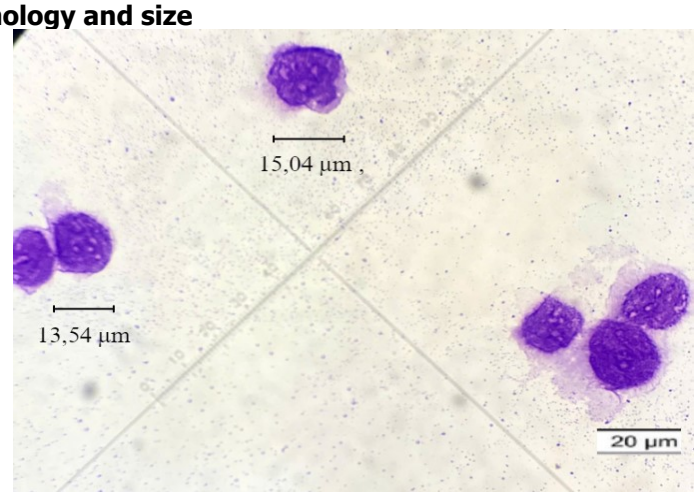


**Figure 3.1.** Lymphoblast morphology on Giemsa-stained slides. Cells appear round, with prominent nuclei and thin cytoplasm. Scale bar = 20 µm.

### 3.3. Lymphoblast culture results after 12 days

**Table 3.2.** Lymphoblast counts after culture at various time points

| Sample | Day 1 (×$10^6$ cells) | Day 3 (×$10^6$ cells) | Day 6 (×$10^6$ cells) | Day 9 (×$10^6$ cells) | Day 12 (×$10^6$ cells) | Fold Expansion (Day12/Day1) |
|---|---|---|---|---|---|---|
| MS.01 | 5.94 | 8.16 | 19.50 | 135.98 | 221.00 | 37.20 |
| MS.02 | 5.40 | 7.02 | 18.90 | 123.12 | 198.99 | 36.85 |
| MS.03 | 4.68 | 6.25 | 15.88 | 107.03 | 173.92 | 37.16 |
| **Average** | 5.34 | 7.14 | 18.09 | 122.04 | 197.97 | 37.07 |

Peripheral blood lymphocytes successfully transformed into lymphoblastoid cells, exhibiting robust proliferation over 12 days (**Table 3.2**). The average cell count **increased ~37-fold**, from 5.34 × $10^6$ on day 1 to 197.97 × $10^6$ on day 12. Individual samples showed minor variation, with MS.01 reaching the highest expansion (221 × $10^6$, 37.20-fold) and MS.03 the lowest (173.92 × $10^6$, 37.16-fold), indicating reproducible growth across donors.

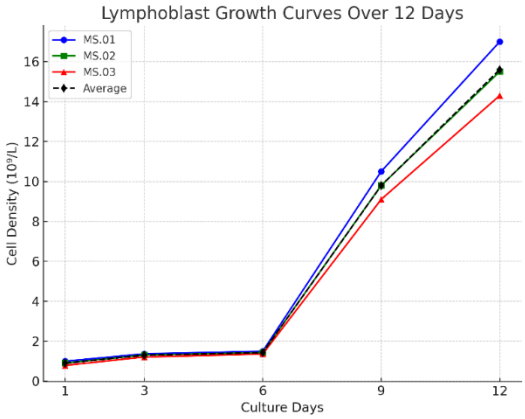


**Figure 3.2.** Growth curves of lymphoblasts over 12 days of culture.
Individual samples (MS.01–MS.03) are shown as colored lines; the mean of three samples is indicated by a dashed black line. Rapid proliferation occurs after day 6, entering the logarithmic phase.

Growth kinetics (**Figure 3.2**) revealed a lag phase until day 6, with moderate cell densities (0.89–1.42 × $10^9$/L), followed by a rapid logarithmic expansion from day 6 to day 12, reaching 15.6 × $10^9$/L. All samples exhibited similar trends, confirming consistent EBV-induced proliferation.

### 3.4. Lymphoblast viability

Cell viability was assessed using the Trypan Blue exclusion assay after 12 days of culture. Total and viable cell counts are summarized in **Table 3.3**.

**Table 3.3.** Lymphoblast viability after 12 days of culture

| Sample | Total Cells (×$10^6$/mL) | Viable Cells (×$10^6$/mL) | Viability (%) |
|---|---|---|---|
| MS.01 | 17.00 | 15.67 | 92.17 |
| MS.02 | 15.52 | 13.83 | 89.13 |
| MS.03 | 14.31 | 12.90 | 90.15 |
| **Average** | 15.61 | 14.13 | 90.48 |

Viability, assessed by Trypan Blue exclusion, averaged 90.48% (89.13–92.17%), exceeding the typical 80–90% for EBV-transformed cells. This may reflect optimized culture conditions or enhanced cell selection during isolation (**Table 1, Figure 3.3**).

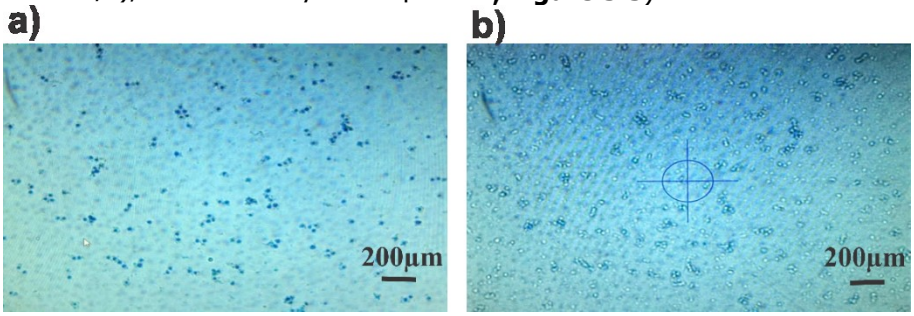


**Figure 3.3.** Lymphoblast viability assessed by trypan blue exclusion.
**(a)** Dead lymphoblasts exhibiting compromised membranes stained dark blue with Trypan Blue.
**(b)** Live lymphoblasts excluding Trypan Blue, displaying intact membranes and bright appearance.
Scale bar = 200 µm.

### 3.5. Quality control

Culture integrity was confirmed throughout the 12-day lymphoblast expansion. No bacterial or fungal contamination was observed, as indicated by the clear appearance of culture medium and microscopic examination of Giemsa-stained slides. Furthermore, cell proliferation and viability were consistent across all samples, with minimal variation, supporting the reproducibility and reliability of the culture protocol.

## IV. DISCUSSION

This study establishes a standardized protocol for isolating, transforming, and culturing lymphoblasts from peripheral blood, providing a robust foundation for research in immunology, genetics, metabolic disorders, and cancer vaccines. Initial white blood cell (WBC) and lymphocyte counts (4.90–7.95 × $10^9$/L and 1.82–3.22 × $10^9$/L, respectively) were within normal ranges for healthy adults,[1] with lymphocyte percentages (34.7–40.5%) consistent with global standards.[2] The higher lymphocyte count in MS.01 (3.22 × $10^9$/L) versus MS.03 (1.82 × $10^9$/L) highlights the importance of sample selection for effective PBMC isolation using Ficoll-Hypaque centrifugation.[15]

The separation process achieved an average lymphocyte purity of 89% post-Ficoll-Hypaque treatment, aligning with previous reports of 85–

95% purity.[15] Consistent yields (0.78–0.99 × $10^9$/L) underscore the technique's reproducibility, critical for subsequent EBV transformation.[6] Morphological analysis via Giemsa staining revealed typical lymphoblast features-round cells with large, dark nuclei (80–90% of volume) and thin cytoplasm-with over 90% normal morphology, consistent with EBV-transformed characteristics.[6]

Proliferation over 12 days yielded a mean fold expansion of 37.07 (range 36.85–37.20),[6] with logarithmic growth post-day 6, reaching 15.60 × $10^9$/L, suitable for large-scale studies. Viability averaged 90.48% (89.13–92.17%) via Trypan Blue exclusion,[14] aligning with reported ranges of 80–90% for EBV-transformed cells,[2] reflecting optimal culture conditions. Quality control measures, including sterile handling and timely processing, ensured integrity and reproducibility.[8]

This protocol provides a reliable platform for advancing research in immunology, genetics, metabolic disorders, and cancer vaccines, enhancing biotechnology applications.

## V. CONCLUSION

This study successfully establishes a standardized protocol for isolating, transforming, and culturing lymphoblasts from peripheral blood, providing a robust platform for advancing scientific research. The method, integrating Histopaque-Ficoll separation and Epstein-Barr virus (EBV)-induced transformation, achieved high lymphocyte purity (up to 90%), robust proliferation (mean fold expansion of 37.07 over 12 days), and excellent viability (mean 90.48%). These results demonstrate reproducibility and reliability across varying sample qualities, affirming the protocol's effectiveness. The optimized procedures support a consistent cell source, essential for future investigations in immunology, genetics, metabolic disorders, and cancer vaccine development. By establishing a reliable framework, this study enhances the scientific foundation for exploring disease mechanisms and therapeutic strategies, contributing to the advancement of biotechnology research.

## REFERENCES


1. **Orkin SH, Zon LI.** Hematopoiesis: an evolving paradigm for stem cell biology. *Cell*. 2008;132(4):631-644. doi:10.1016/j.cell.2008.01.025
2. **Longo N.** Primary carnitine deficiency and newborn screening for disorders of the carnitine cycle. *Ann Nutr Metab*. 2016;68(suppl 3):5-9. doi:10.1159/000448321
3. **Longo N, Amat di San Filippo C, Pasquali M.** Disorders of carnitine transport and the carnitine cycle. *Am J Med Genet C Semin Med Genet*. 2006;142C(2):77-85. doi:10.1002/ajmg.c.30087
4. **Tran TCM, Ta VT, Bui TB, Vu CD, Pham TN.** A case study of lethal neonatal CPT II deficiency: novel insights from genetic analysis. *Mol Genet Metab Rep*. 2024;41:101170. doi:10.1016/j.ymgmr.2024.101170
5. **El-Hattab AW, Li FY, Shen J, et al.** Maternal systemic primary carnitine deficiency uncovered by newborn screening: clinical, biochemical, and molecular aspects. *Genet Med*. 2010;12(1):19-24. doi:10.1097/GIM.0b013e3181c5e6f7
6. **Omi N, Tokuda Y, Ikeda Y, et al.** Efficient and reliable establishment of lymphoblastoid cell lines by Epstein-Barr virus transformation from a limited amount of peripheral blood. *Sci Rep*. 2017;7:43833. doi:10.1038/srep43833
7. **Magoulas PL, El-Hattab AW.** Systemic primary carnitine deficiency: an overview of clinical manifestations, diagnosis, and management. *Orphanet J Rare Dis*. 2012;7:68. doi:10.1186/1750-1172-7-68
8. **Golke T, Mucher P, Schmidt P, et al.** Delays during PBMC isolation have a moderate effect on yield, but severely compromise cell viability. *Clin Chem Lab Med*. 2022;60(5):701-706. doi:10.1515/cclm-2022-0003
9. **Jaatinen T, Laine J.** Isolation of mononuclear cells from human cord blood by Ficoll-Paque density gradient. *Curr Protoc Stem Cell Biol*. 2007;Chapter 2:Unit 2A.1. doi:10.1002/9780470151808.sc02a01s1
10. **Yao T, Asayama Y.** Animal-cell culture media: history, characteristics, and current issues. *Reprod Med Biol*. 2017;16(2):99-117. doi:10.1002/rmb2.12024
11. **Nguyễn Quý Linh, Tạ Thành Đạt, Lê Văn Toàn, Trần Vân Khánh, Hoàng Thị Mỹ Nhung, Trần Huy Thịnh.** Nuôi cấy hoạt hóa, tăng sinh tế bào gamma delta T trên bệnh nhân ung thư phổi. *Tạp chí Khoa học Công nghệ Việt Nam*. 2020;62(2):2.
12. **Böyum A.** Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest Suppl*. 1968;97:77-89. doi:10.3109/00365513.1968.11776185
13. **Feldman AT, Wolfe D.** Tissue processing and hematoxylin and eosin staining. *Methods Mol Biol*. 2014;1180:31-43. doi:10.1007/978-1-4939-1050-2_3
14. **Strober W.** Trypan blue exclusion test of cell viability. *Curr Protoc Immunol*. 2015;111:A3.B.1-A3.B.3. doi:10.1002/0471142735.ima03bs111
15. **Ferrante A, Thong YH.** Optimal conditions for simultaneous purification of mononuclear and polymorphonuclear leucocytes from human blood by the Hypaque-Ficoll method. *J Immunol Methods*. 1980;36(2):109-118. doi:10.1016/0022-1759(80)90036-8