Corning® Lymphocyte Separation Medium (LSM)

Application Note

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Introduction

Lymphocytes are a subtype of white blood cells in the immune system of vertebrates. Natural killer (NK), T, and B cells are three components of lymphocytes responsible for innate and adaptive immunity. Thus, convenient and efficient lymphocyte preparation has extended application in various fields including pathology and adoptive immunotherapy.

Corning Lymphocyte Separation Medium (LSM) is a sterile, ready-to-use reagent designed for the rapid and reliable isolation of peripheral blood mononuclear cells (PBMCs) *in vitro*. It is an iso-osmotic polysucrose and sodium diatrizoate solution with low viscosity based on the adapted method developed by Boyum. It has a density of 1.077 to 1.080 g/mL at 20°C, osmolality of 290 ± 20 mOsm, and pH of 7.5 ± 1.5; sodium hydroxide can be added to adjust the pH when necessary.

LSM is manufactured under an ISO 13485 certified quality system in an FDA registered cGMP facility. Each product is sterile-filtered and released only when all the stringent quality criteria are met. Thus, it is an endotoxin-free reagent to prevent any risk of contamination and irregular cellular functions. This product is provided in 100 mL and 500 mL volumes (Corning Cat. Nos. 25-072-CI and 25-072-CV, respectively), and can be packaged to suit customer requirements.

LSM relies on centrifugation to form multiple layers of different cell types. Erythrocytes and granulocytes sediment to the bottom of the tube and constitute the major content of the pellet. Mononuclear cells (lymphocytes and monocytes) remain at the interphase of the plasma-LSM and can be collected by discarding the upper plasma layer and aspirating the cells at the interface to a new tube. Remaining platelets, LSM, and plasma can be removed by additional washing steps.

In this study, Corning LSM and a Competitor product are compared in parallel. Diluted whole blood treated with an anti-coagulant agent was used for lymphocyte separation. EDTA, which contributes to mononuclear purity, was also analyzed. The major criteria for this assay were cell number and cell viability.

Materials and Methods

Blood Sample

Fresh blood was provided by healthy donors using a BD Vacutainer® tube that contained the anti-coagulant Heparin (BD Cat. No. 367884). The sample was drawn within 2 hours to ensure high reliability of isolated PBMCs; blood laid aside for more than 24 hours should be excluded.

Lymphocyte Separation

After receiving the fresh blood sample, subsequent lymphocyte separation steps were implemented as soon as possible:

- Corning LSM (Corning Cat. No. 25-072-CI) and Competitor lymphocyte separation media were warmed to room temperature before use. Both separation media were gently inverted to ensure thorough mixing.
- 3 mL of Corning LSM and Competitor product were aseptically transferred to a 15 mL Corning CentriStar™ tube (Corning Cat. No. 43079).
- Blood samples were diluted with an equal volume of PBS without calcium and magnesium (Corning Cat. No. 21-040-CVR) or other balanced salt solutions, such as DPBS (Corning 21-031-CVR) or Hank's Balanced Salt Solution (Corning 21-021-CVR). Another sample diluted with PBS containing 2 mM EDTA for comparison was prepared.
- 4 mL of diluted blood sample were carefully layered (not mixed) on top of the separation medium solution, and a sharp interphase between the medium and blood sample was observed.
- Tubes were centrifuged at 400 xg for 30 minutes at room temperature.

Note: The brake of the centrifuge should be turned off (set the deceleration to zero, as quick deceleration will disturb the gradient). This two-phase system was gradually divided into several layers during this process.

- The top layer of clear plasma was aspirated to within 1 cm above the lymphocyte layer and discarded.
- The lymphocyte layer (including 1 cm solution above and below the interface) was transferred to a new centrifuge tube (approximately 2 mL).
- The transferred lymphocytes were then diluted with at least 5 volumes of balanced salt solution and mixed gently. They were centrifuged at 500 xg for 10 minutes at room temperature.

- The supernatant, which mainly contains the separation medium and platelets, was removed.
- Cells were washed 2 more times with PBS buffer at 160 *g* to 260 *g* for 10 minutes at room temperature.
- The cell pellet was re-suspended in the appropriate medium for further application.

Cell Yield

Cell number and cell viability of isolated lymphocytes were determined using Cell Countess® Automated Cell Counter (Thermo Fisher). Cells were stained with 0.4% Trypan Blue (Thermo Fisher Cat. No. T10282) and mixed thoroughly.

We then added a 10 µL stained sample into the chamber of the cell counting slides (Thermo Fisher Cat. No. C10283) and counted the cell number following the manufacturer's instructions.

Statistical Analysis

Statistical analysis was conducted using Student's t-test to compare the difference of cell yield between each experimental group.

Results

Samples were divided into 4 groups based on the purpose of this analysis:

- Corning® LSM with EDTA
- Corning LSM without EDTA
- Competitor separation medium with EDTA
- Competitor separation medium without EDTA

Each experimental group was independently tested in triplicate, and the cell number of each sample was counted twice.

The comparison of lymphocyte yield using different separation media is shown in Figure 1A. No significant difference was found between Corning® LSM and Competitor products, no matter with EDTA (p = 0.17) or without EDTA (p = 0.68).Therefore, additional EDTA did not increase the finally obtained lymphocytes (p >0.05). For all the experimental groups, the cell viability was higher than 90% (Figure 1B), and no significant difference was found between each tested group (p >0.05). Overall, Corning LSM showed equal performance with the tested Competitor product.

Conclusion

- Corning Lymphocyte Separation Medium enabled the rapid and reliable separation of lymphocytes from anti-coagulated fresh human blood samples.
- Corning Lymphocyte Separation Medium yields approximate 95% mononuclear cells present in the original blood sample.
- The cell viability of separated lymphocytes by Corning Lymphocyte Separation Medium was above 90%.

References

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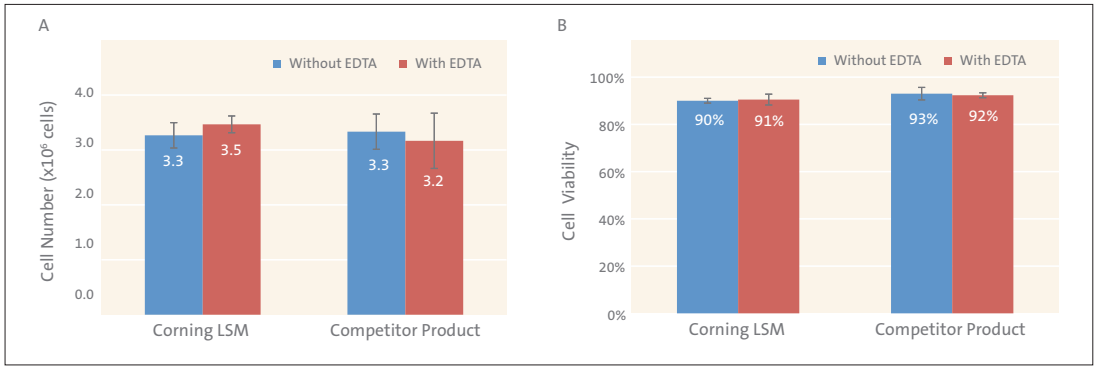

Figure 1. Cell yield of lymphocytes isolated by different separation media. Average cell number and viability of Corning LSM and Competitor product with, and without, extra EDTA were calculated in triplicate. No significant difference (p >0.05) was found between each group.

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