USER MANUAL

LSM™ Lymphocyte Separation Medium

Cat. No. 0850494, 0850494X

Revision Date: 2024-03

INTENDED USE

For in vitro isolation of mononuclear cells (lymphocytes) from peripheral blood and other blood sources.

SUMMARY OF THE METHOD

Early methods for isolating lymphocytes involved mixing blood with a compound which aggregated the erythrocytes, but only slightly affected lymphocytes. With centrifugation, erythrocytes pelleted due to their increased density and leukocytes were collected from the upper portion of the tube.[1]

Boyum[2] introduced a more convenient and rapid separation using centrifugation through a Ficoll®-sodium metrizoate solution. Diluted blood was layered over the Ficoll®-sodium metrizoate solution and centrifuged at 400 x g for 30 minutes. Erythrocytes and granulocytes sedimented to the bottom of the tube, and lymphocytes and platelets were collected from the interface between the two phases.

Modifications to the Boyum formulation have been made by numerous researchers.[3-6] LSM, manufactured by MP Bio, has a unique formulation using the successful substitution of sodium diatrizoate for sodium metrizoate.

PRINCIPLE OF THE PROCEDURE

Defibrinated or heparinized blood is diluted with a balanced salt solution, such as PBS or HBSS at a 1:1 ratio, layered over the separation medium and centrifuged at 400 x g for 30 minutes. During centrifugation, differential migration results in the formation of several cell layers.

A pellet is formed that is comprised mainly of erythrocytes and granulocytes which have migrated through the gradient. Due to their density, lymphocytes and other mononuclear cells (platelets and monocytes) are found at the plasma-LSM interface. Lymphocytes are recovered by aspirating the layer. Further washing removes platelets, LSM and plasma.

REAGENTS

LSM is a sterile, filtered solution containing Polysucrose 400 and sodium diatrizoate at a density of 1.0770 - 1.0800 g/mL at 20 °C.

STORAGE

- Store at room temperature (15-30 °C) in the original container.
- Stable until the expiration date listed on the vial.
- Protect from light.

RECOMMENDED PRODUCTS

- RPMI 1640 (1X) with L-Glutamine (Cat. No. 0912603)
- RPMI 1640 with L-glutamine (powder) (Cat. No. 0910601)
- Hank's Balanced Salts (Cat. No. 091810554)
- DPBS (1X, Cat. No. 091860454)

CAUTION

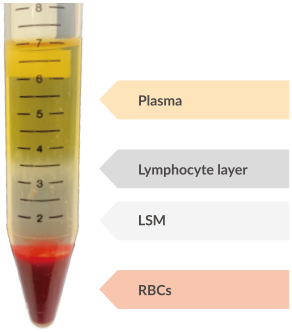
- The material is intended for laboratory use only for the in vitro separation of lymphocytes from peripheral blood.
- LSM is not intended for in vivo use.
- Do not use if the material is cloudy, has a distinct yellow color or shows any signs of contamination.

INSTRUCTIONS FOR USE

The following is one of many variants of the procedure originally described by Boyum[2]. This procedure was developed for use with defibrinated or anti-coagulant treated blood. For optimal results, alterations may be necessary depending on the species the blood or tissue was collected from.

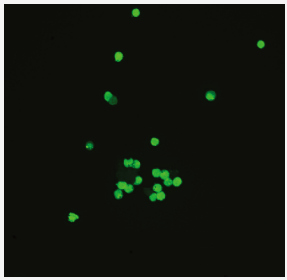
1. Thoroughly mix the LSM by inverting the bottle gently.
2. Aseptically transfer 3 mL of LSM to a 15 mL centrifuge tube.
3. Dilute 2 mL of defibrinated or heparinized blood with 2 mL of DPBS with no Mg^{2+} or Ca^{2+}. 0.9% NaCl, HBSS or cell culture media can also be used as a diluent.
4. Carefully layer the diluted blood over 3 mL of LSM (room temperature) in a 15 mL centrifuge tube, creating a sharp blood-LSM interface. Do not mix diluted blood with the LSM.
5. Centrifuge the tube at 400 x g at room temperature for 15-30 minutes with the brake off. Centrifugation should sediment erythrocytes and polynuclear leukocytes and band lymphocytes above the LSM.
6. Aspirate the top layer of clear plasma to within 2-3 mm above the lymphocyte layer.
7. Aspirate the lymphocyte layer plus about half of the LSM layer below it and transfer it to a new centrifuge tube. Add an equal volume of DPBS to the mononuclear cell layer in the centrifuge tube and centrifuge for 10 minutes at room temperature at 200 x g (a centrifugation speed ranging from 160-260 x g can be used–choose a speed sufficient to sediment the cells without damage). Note that washing removes LSM and reduces the percentage of platelets.
8. Wash the cells again with DPBS and resuspend in the appropriate medium for your applications.

NOTE ▸ Erythrocytes may be present in *the monoclear fraction at 1-5% of total* cells counted. They can be removed using *a red blood cell lysis solution if necessary.*



Lymphocyte separation schematic using LSM

RESULTS ▸



Bovine lymphocytes (with erythrocytes removed) were obtained using MP Biomedicals LSM reagent according to the manufacturer's protocol. Live/Dead analysis of isolated cells was performed using the LIVE/DEAD Viability/ Cytotoxicity assay kit from Molecular Probes. 95% of lymphocytes recovered from 2 mL of whole heparinized bovine blood were LIVE. Fluorescent microscopy of cells shows LIVE cells in green (40x magnification).

REFERENCES

[1] Boyum, A. Separation of white blood cells. *Nature*. **1964**, *204*, 793-794.

[2] Boyum, A. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest. Suppl*. **1968**, *97*, 77.

[3] Harris, R.; Ukaejiofo, E. V. Rapid preparation of lymphocytes for tissue typing. *Lancet*. **1969**, *2*, 327.

[4] Thorsby, E.; Bratlie, A. A rapid method for preparation of pure lymphocyte suspensions. *In Histocompatibility Testing. P.I. Terasaki, ed. Munksgaard, Copenhagen*. **1970**, 664-665.

[5] Ting, A.; Morris, P.J. A technique for lymphocyte preparation from stored heparinized blood. *Vox Sang*. **1971**, *20*, 561.

[6] Wang, W.; et al. Zika virus infection induces host inflammatory responses by facilitating NLRP3 inflammasome assembly and interleukin-1β secretion. *Nat. Commun*. **2018**, *9*, 106.

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