Lymphoprep™

Density Gradient Medium for the Isolation of Mononuclear Cells

Catalog #07801	250 mL
Catalog #07811	4 x 250 mL
Catalog #07851	500 mL
Catalog #07861	6 x 500 mL

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Product Description

Lymphoprep™ is a density gradient medium recommended for the isolation of mononuclear cells (MNCs) from peripheral blood, cord blood, and bone marrow by exploiting differences in cell density. Granulocytes and erythrocytes have a higher density than MNCs and therefore sediment through the Lymphoprep™ layer during centrifugation. Lymphoprep™ can be substituted for Ficoll-Paque™ without any need to change existing protocols and is fully compatible with both SepMate™ and RosetteSep™.

This method has been found to be rapid, simple and reliable, and gives excellent results with blood samples from most normal individuals and patients.

SEPARATION PRINCIPLE:

Differences in cell density are exploited to separate granulocytes and erythrocytes from MNCs. Granulocytes and erythrocytes have a higher density at the osmotic pressure of Lymphoprep™, and they sediment through the Lymphoprep™ layer during centrifugation. The polysaccharide in Lymphoprep™ enhances erythrocyte aggregation, thereby increasing erythrocyte sedimentation. MNCs, with lower densities, remain at the plasma:Lymphoprep™ interface.

Properties

Storage: Store at 15 - 25°C.

Shelf Life: Stable until expiry date (EXP) on label.

Protect product from light. Prolonged exposure to direct sunlight leads to release of iodinine from the sodium diatrizoate molecule. This effect is negligible when working with this solution on a day-to-day basis.

Handling / Directions for Use

1. Mix Lymphoprep™ thoroughly before use by inverting the bottle several times.
2. Add Lymphoprep™ to tube (see Table 1).
3. Dilute blood with an equal amount of phosphate-buffered saline plus 2% fetal bovine serum (PBS + 2% FBS; Catalog #07905), or other suitable culture medium.
4. Layer blood on top of Lymphoprep™, being careful to minimize mixing of blood with Lymphoprep™.
5. Centrifuge at 800 x *g* for 20 minutes at room temperature (15 - 25°C) with brake off. If the blood has been stored for more than 2 hours, increase the centrifugation time to 30 minutes.
6. Remove and discard upper plasma layer without disturbing the plasma:Lymphoprep™ interface.
7. Remove and retain MNC layer at the plasma:Lymphoprep™ interface without disturbing erythrocyte/granulocyte pellet.
8. Wash MNCs once with medium.

Table 1: Recommended Volumes and Tube Sizes

BLOOD (mL)	PBS + 2% FBS (mL)	LYMPHOPREP™ (mL)	TUBE SIZE (mL)
1	1	1.5	5
2	2	3	14
3	3	3	14
4	4	4	14
5	5	10	50
10	10	15	50
15	15	15	50

Notes and Tips

- Erythrocyte contamination in the mononuclear cell fraction is usually 1 - 5% of the total cell number. Some immature granulocytes may also be collected at the interface if a patient is undergoing intense immunosuppressive therapy.
- When heparinized blood is used, it is essential to remove most of the platelets, in order to avoid inhibition in the cytotoxicity test. The described washing procedure is usually sufficient (step 8 in Handling/Directions For Use).

References

Böyum A. (1968) Isolation of leucocytes from human blood. Further observations. Methylcellulose, dextran, and ficoll as erythrocyteaggregating agents. Scand J Clin Lab Invest Suppl 97: 31–50.

Böyum A. (1968) Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand J Clin Lab Invest Suppl 97: 77–89.

Böyum A. (1964) Separating of White Blood Cells. Nature 204: 793–4.

Thorsby E. & Bratlie A. (1970) A rapid method for preparation of pure lymphocyte suspensions. In P.Terasaki, ed. Histocompatibility Testing. Munksgaard Copenhagen, pp. 655–6.

Ting A & Morris PJ. (1971) A technique for lymphocyte preparation from stored heparinized blood. Vox Sang 20(6): 561–3.