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, reliable, and gives excellent results with blood samples from most normal individuals and patients.

Lymphoprep™ is a sterile, endotoxin-tested solution that is manufactured according to GMP and ISO 13485.

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 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 4 - 30°C.

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

Contains:
- Sodium diatrizoate (9.1% w/v)
- Polysaccharide (5.7% w/v)
- Other ingredients

Sterility: Autoclaved for 15 minutes at 121°C.

Density: 1.077 g/mL

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.

Directions for Use

1. Warm Lymphoprep™ to room temperature (15 - 25°C) before use.
2. Remove the bottle cap and mix thoroughly by inverting the bottle several times.
3. Withdraw Lymphoprep™ using either a syringe or a pipettor, as described below.

   Syringe:

   With the bottle in the inverted position, insert the syringe needle through the rubber septum and withdraw desired volume.

   Pipettor:

   a. Lift the aluminum ring and pull off the metal seal. Remove the silver ring.
   b. Using aseptic technique, remove the rubber septum and withdraw the required volume.
   c. Using aseptic technique, reinsert the rubber septum.
4. Add Lymphoprep™ to tube (see Table 1 for recommended volumes).

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

5. Dilute blood with an equal amount of Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum (PBS + 2% FBS; Catalog #07905), or other suitable culture medium.
6. Layer blood on top of Lymphoprep™, being careful to minimize mixing of blood with Lymphoprep™.
7. 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.
8. Remove and discard upper plasma layer without disturbing the plasma:Lymphoprep™ interface.
9. Remove and retain the MNC layer at the plasma:Lymphoprep™ interface without disturbing the erythrocyte/granulocyte pellet.
10. Wash MNCs once with medium.

Related Products

For related products including SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) tubes for facilitated cell isolation using density gradient centrifugation, cell isolation kits, antibodies, and other density gradient media, visit www.cellseparation.com or contact us at techsupport@stemcell.com.

*SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions SepMate™ is available for research use only (RUO).

Notes and Tips

- Erythrocytes may be present in the mononuclear cell fraction at 1 - 5% of the total cell number.
- If working with blood from patients undergoing immunosuppressive therapy, some immature granulocytes may be collected at the plasma:Lymphoprep™ interface.

References

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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.