Leucosure

Catalog No- EXP002

Store at 2°C to 8°C. Away from direct light.

Product Description

Leucosure is a human lymphocyte separation medium which is sterile, endotoxin tested and ready-to-use solution. This product contains polysucrose, which accelerates erythrocyte precipitation, helps to improve the separation result of lymphocytes. The lymphocytes isolated are available for the cell culture and cytotoxicity assays. This product is mainly used for the separation of human peripheral blood lymphocytes, but also for the separation of most mammalian mononuclear cells.

Product Contents

Catalog No	Volume	Storage
EXP001-50ML	50ml	2-8˚C
EXP001-100ML	100ml	2-8˚C

Principle

The density of mononuclear cells (lymphocytes and monocytes) in peripheral blood is between 1.075-1.090g/ml, which is different from that of red blood cells, multinuclear leucocytes and platelets. The densities of red blood cells and granulocytes are relatively large (about 1.092g/ml) and the density of platelets is between 1.030~1.035g/ml. Therefore, Leucosure is used to generate a certain degree of density gradient, and the diluted whole blood is smoothly paved on the separation medium. After centrifugation, the red blood cells and granulocytes sink to the bottom of the tube due to their large density; Since their densities are less than or equal to the density of separation medium, lymphocytes and monocytesare found at the surface of separation medium. There may also be a small amount of cells suspended in the separation medium. By sucking the cells on the surface of separation medium, mononuclear cells can be separated from peripheral blood.

Product Details

- Endotoxin < 0.5EU/mL
- Density (1.077±0.001)g/mL (20°C)
- Osmolality (290±15)mOsmol/kg.
- >95% viability of the separated cells as determined by trypan blue exclusion staining
- For best results, bring the solution to room temperature (15-25°C) before use, and invert the bottle several times.
- This product has an expiration period of 2 years.
- Do not use, if the material is cloudy, has a distinct yellow color, or shows any sign of contamination

Protocol

1. Fresh anticoagulant (EDTA, sodium citrate or heparin and other anticoagulants are acceptable) whole blood is taken. Dilute the whole blood with an equal volume of isotonic solution (PBS or normal saline).
2. Add a certain volume of separation medium to the centrifuge tube. Carefully layer the diluted blood sample over the separation medium, keep clear interface between the two liquid surfaces. The volume ratio of separation medium, anticoagulant undiluted whole blood and isotonic solution (PBS or normal saline) is 1:1:1.
3. Centrifuge at 700-800g on a swing rotor for 20-30 mins at room temperature. The acceleration and deceleration are set to a slower speed.
4. After centrifugation, the red blood cells are at bottom of the tube, the separation medium is at the middle layer, and the top layer is the plasma/tissue homogenate layer. A thin and dense white membrane found at the plasma-separation medium interface is the mononuclear cell layer (including lymphocytes and monocytes).Transfer the white membrane layer into another centrifuge tube carefully.
5. Diluted the mononuclear cell layer to a certain volume with isotonic solution (PBS, normal saline or medium, etc.), then shake to mix well. Centrifuged at 250g on a swing rotor for 10min at room temperature, then remove the supernatant solution. Wash the cells once or twice.
6. Resuspend the cells with isotonic solution (PBS, normal saline or medium, etc.) and count the cells for later use.

*Notes

1. The isotonic medium (PBS or 0.9% NaCl) used to dilute anticoagulated blood should be sterile, and can be replaced by RPMI-1640 medium.

2. 1:1 blood dilution can reduce the coagulation of erythrocytes and increase the amount of lymphocytes harvested.

3. For the activity of lymphocyte, blood should be separated immediately after collection. The cells harvested are peripheral blood mononuclear cells (PBMCs), including lymphocytes and monocytes.

4. Long storage of blood may cause bad result that there are more residual RBCs in the harvested lymphocytes. The time of centrifugation should be extended to improve the result.

5. If the procedures are correct but there is no layer of PBMCs found at the interfaces after centrifugation, make sure that the blood is enough and fresh, and the separation medium is suitable for the blood of this species animal.

6. Once opened, please store the separation medium at 2-8°C to avoid the change of density of the separation solution caused by liquid volatilization, which will affect the separation effect.

7. Prevent contamination of the reagent.

8. Purity of the cell population can be determined by automation or by performing Romanowsky staining (Wright staining) on a cytospin slide prepared from cells collected in Step 6. Slide preparation can be done by air drying the cell suspension obtained in the final step.

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References

1. Bøyum, A "Isolation of mononuclear cells and granulocytes from human blood ." Scand.J.Clin.Lab.

2. EC Guide to GMP (Good Manufacturing Practice), annex 1 “Manufacture of Sterile Medicina Products”.

3. Bøyum, A Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. 21, Suppl. 97 (Paper IV), 77–89 (1968).

Technical Assistance

For any kind of technical assistance, mail to info@exsure.in or Call 9836956514