Lymphocyte Separation Medium 1.077
Catalog No:RC0010

Product Information

EnkiLife Lymphocyte Separation Medium (LSM) is a ready-to-use separation medium that has been filter-sterilized. It is suitable for the in vitro isolation of human peripheral blood lymphocytes and can also be used to isolate lymphocytes from other tissues, including umbilical cord blood and bone marrow cells.
Each 100 mL of LSM contains 6.2g of polysucrose and 9.4g of sodium diatrizoate. The density of LSM is 1.0770-1.0800 g/mL at 20℃.

Principle

The density of red blood cells and granulocytes in human blood is approximately 1.090 g/mL. Lymphocytes and monocytes have a density of 1.075–1.090 g/mL, while platelets have a density of 1.030–1.035 g/mL. By using defibrinated or heparin-treated blood, diluted 1:1 with normal saline or balanced salt solution, and performing low-speed centrifugation for 30 minutes using a density gradient solution with a density between 1.075 and 1.092 (nearly isotonic), cells of specific densities will distribute according to the density gradient, thereby separating different types of blood cells.
Red blood cells and polymorphonuclear granulocytes migrate through the gradient and settle at the bottom of the tube. Lymphocytes and other mononuclear cells (monocytes and platelets) are distributed between the plasma and the LSM layers. Lymphocytes can be recovered by aspirating the interphase solution and then further washed to remove platelets, LSM, and plasma.

Size

200mL/500mL/1000mL

Storage

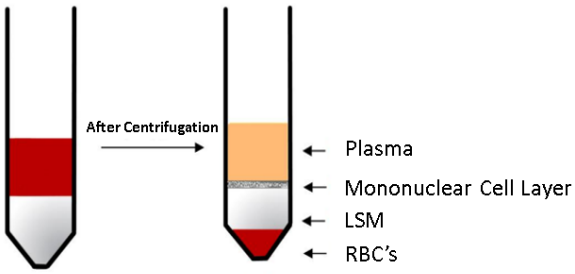
Stored at 15~30℃, valid for 24 months.

Quality inspection standards

1. Ficoll-based solution with a density of 1.077 g/mL.
2. Strict sterility, mycoplasma, endotoxin tests are carried out to ensure that the product does not contain bacteria, fungi, mycoplasma and viruses.
3. Purpose: Isolation of mononuclear cell fraction from human peripheral blood.

Operation steps

1. Gently invert the bottle to thoroughly mix the LSM.
2. Under sterile conditions, transfer 3mL of LSM into a 15mL centrifuge tube.
3. Mix 2mL of defibrinated or heparinized blood with 2mL of normal saline.
4. Carefully layer the diluted blood on top of the 3mL LSM (at room temperature) in the 15 mL centrifuge tube, forming a distinct interface between the blood and LSM. Do not mix the diluted blood into the LSM.
5. Centrifuge at room temperature at 400×g for 15–30 minutes. Centrifugation will precipitate red blood cells and polymorphonuclear leukocytes, while forming a mononuclear lymphocyte layer on top of the LSM, as shown in the figure below (from top to bottom: plasma layer, mononuclear cell layer, LSM layer, RBC pellet).



6. Aspirate 2–3 mm of the plasma above the lymphocyte layer.
7. Carefully remove the lymphocyte layer and the lower half of the LSM layer, and transfer them to another centrifuge tube. Add an equal volume of balanced salt buffer to the lymphocyte layer in the tube, and centrifuge at room temperature (18–25°C) for 10 minutes at a speed that will pellet the cells without causing damage, such as 160–260×g (to wash away the LSM and reduce the percentage of platelets).
8. Wash the cells again with balanced salt buffer, and finally resuspend the cells in an appropriate culture medium (as required by the experimenter).

Notes

1. For your safety and health, please wear a lab coat and disposable gloves during the procedure.
2. The normal saline used to dilute defibrinated or heparinized blood must be sterile.
3. To maintain the viability of lymphocytes, separation should be performed as soon as possible after blood collection. The isolated cell layer is actually the mononuclear cell layer, which includes lymphocytes and monocytes.
4. This product is For Research Use Only, Not for Diagnostic Use.