DATA SHEET

Technical Data Sheet

Date: 28/4/2006

Lymphosep, Lymphocyte Separation Media
CAT N: LM-T1702

Theoretical pH:	7.0 ± 0.5
Colour:	Colourless, clear solution
Storage conditions:	Room temperature
Shelf life:	24 months
Sterility tests:	Bacteria in aerobic and anaerobic conditions Fungi and yeast
Endotoxin:	< 10 EU/ml (<1ng/ml)

Recommended use:
Use aseptic technique when handling this product. For in vitro laboratory use only, not for drug, human or veterinary use.

Application:
Lymphosep is degigned for the simple, rapid isolation of lymphocytes from whole blood that has been diluted and treated with anti-coagulant or defrinating agent.
For best results use blood drawn less than 2 hours before Do not use blood more than 24 hours from when it was drawn.

Uses:

1) Thoroughly mix the Lymphosep by inverting the bottle gently.
2) Aseptically transfer 3ml of Lymphosep to a 15ml centrifuge tube
3) Mix 2ml of defibrintated or hepranised blood with 2ml of physiological saline (PBS w/o ca w/o Mg) or balanced salt solution (LM-S2041)
4) Carefully layer the diluted blood over 3ml of Lymphosep (room temperature) in a 15ml centrifuge, creating a sharp blood-Lymphosep interphase. DO NOT MIX! The quality of the separation is dependant upon a sharp interphase between the lymphocytes and the solution.
5) Centrifuge the tube at 400g at room temperature for 15 to 30 minutes. Centrifugation should sediment erythrocytes and polynuclear leukocytes and band mononuclear lymphocytes above the lymphosep.
6) Aspirate the top layer of clear plasma to within the 2-3mm above the lymphocyte layer.
7) Aspirate the lymphocyte layer plus about half the lymphosep layer below it and transfer it to a centrifuge tube. Add an equal volume of buffered balanced salt solution to the lymphocyte later in the centrifuge tube and centrifuge for 10 minutes at room temperature (18-25°C) at a speed sufficient to sediment to cells without damage i.e. 160-260g. Washing the cells removes Lymphosep and reduces the percentage of platelets.
8) Wash the cells again with a buffered balanced sale solution (LM-S2041) and re-suspend in the appropriate medium for your application.

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