www.lonza.com
U.S. Scientific Support: 800-521-0390
scientific.support@lonza.com
EU/ROW Scientific Support: +49-221-99199-400
scientific.support.eu@lonza.com
Document # INST-17-829-2 09/11

Lymphocyte Separation Medium

Instruction for Use

17-829E

Introduction

Lymphocyte Separation Medium is a mixture of Ficoll® and sodium diatrizoate (Hypaque) with density adjusted to 1.077 g/ml. This sterile filtered product is intended for laboratory/manufacturing use, and is not for *in vitro* diagnostic use. It is commonly used to isolate lymphocytes from human blood. One protocol to accomplish this is presented here.

Protocol

1. Anticoagulated blood (citrated or heparinized) should be used.

   NOTE: Always treat human and other primate source material as potentially infectious and take safety precautions.

2. Dilute blood 1:1 with calcium-magnesium-free PBS and layer 9 ml onto 6 ml Lymphocyte Separation Medium. Use a clear plastic centrifuge tube with a cap. For large volumes use a similar ratio of diluted blood to Lymphocyte Separation Medium.
3. Centrifuge at 400 x g for 15 minutes.
4. Remove plasma-PBS without disturbing the interface.
5. Collect the interface with a cannula and dilute to 20 ml in serum-free medium, such as RPMI 1640.
6. Centrifuge at 70 x g for 10 minutes.
7. Discard supernatant fluid and resuspend pellet in 2-3 ml serum-free medium.
8. Count nucleated cells on a hemocytometer or electronic counting device.
9. Lymphocytes will be concentrated at the interface, along with some platelets and monocytes. Granulocytes will be found mostly in the Lymphocyte Separation Medium and erythrocytes will pellet at the bottom of the tube.

References

1. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand. J. Clin. Lab. Invest. Suppl. 97:77-89.
2. Boyum, A. 1976. Isolation of lymphocytes, granulocytes and macrophages. Scand. J. Clin. Lab. Invest. Suppl. 5:9-15.
3. Boyum, A. 1977. Separation of lymphocytes, lymphocyte subgroups and monocytes: a review. Lymphology. 10-2:71-6.
4. Boyum, A. 1984. Separation of lymphocytes, granulocytes and monocytes from human blood using iodinated density gradient media. Methods Enzymol. 108:88-102.
5. Boyum, A. et al. 2002. Separation of Human Lymphocytes from Citrated Blood by Density Gradient (NycoPrep) Centrifugation: Monocyte Depletion Depending upon Activation of Membrane Potassium Channels. Scand. J. Immunol. 56-1:76-84.
6. Koistinen, P. 1987. Human peripheral blood and bone marrow cell separation using density gradient centrifugation on Lymphoprep and Percoll in haematological diseases. Scand. J. Clin. Lab. Invest. 47-7:709-14.
7. Rola-Pleszczynski, M. and W.H. Churchill. 1978. Purification of human monocytes by continuous gradient sedimentation in ficoll. J. Immunol. Methods. 20:255-62.

Product Use Statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.