Bio Bharati Life Science Pvt. Ltd.

Lymphocyte Separation Teaching Kit

Cat# BB-ITK100, Pack Size: 5 reactions

Aim: To separate lymphocyte from whole blood by Density Gradient Centrifugation method.

Principle: A lymphocyte is a small white blood cell, usually 7-8µm in length, present in vertebrate's immune system. Three types of lymphocytes are T cells, B cells and NK cells which can be identified by their large nucleus. Typical process for carrying out lymphocyte separation is the density gradient centrifugation which is the most widely used method for processing of blood.

Separation of lymphocytes from whole blood using HISTOPAQUE-1077 is a commonly used method. This separation medium (HISTOPAQUE-1077) is an aqueous solution of poly-sucrose and sodium diatrizoate whose density is adjusted to ~ 1.077g/ml. This medium facilitates rapid recovery of viable lymphocyte and other mononuclear cells from small volume of blood cells.

Kit Description: This kit enables separation of lymphocyte by using density gradient media HISTOPAQUE- 1077. Sufficient amount of HISTOPAQUE- 1077, diluent buffer and Giemsa's stain have been provided in the kit to carry out 5 experiments.

Duration of experiment: 2hours

Kit Contents:

Sl. No	Items	Quantity for 5 reactions	Store at
1.	Histopaque-1077	14 ml	2-8°C
2.	15 ml Centrifuge Tube	10nos	RT
3.	Microcentrifuge Tube	6nos	RT
4.	Diluent Buffer	50 ml	2-8°C
5.	Wright's Solution	7 ml	RT

Materials required but not provided:

Consumables: 70% Alcohol/Spirit, EDTA coated collection tubes, Cotton, Glass Pasteur pipettes, Microscopic slides, Coverslip.

Instrument: Cold Centrifuge, Microscope

Storage: Bio Bharati Immunoprecipitation Teaching kit is stable for 3 months from the date of receipt without showing any change in performance if stored properly.

Important instructions:

- Read the instructions carefully before starting the experiment.
- Always use freshly anti-coagulated blood while performing the experiment.

Procedure:

Lymphocyte Layer Separation

1. Collect 4ml of blood in the EDTA coated collection tube, using sterile syringe and needle.
2. Mix immediately by inverting the tube 3-5 times.
3. Dilute the blood with diluent buffer provided in the kit in 1:1 ratio i.e. mix 4ml of diluent buffer with 4ml of EDTA
4. Take 2.5 ml of Histopaque-1077 in a new 15ml centrifuge tube. Overlay the Histopaque- 1077 with 7.5ml of diluted blood.
5. Immediately centrifuge at 1600rpm in a Swing Bucket Rotor at 4°C (if Fixed Angle Rotor is used, centrifuge at 2000 rpm)
6. Carefully remove the tube from the centrifuge, observing three layers: top layer is a clear yellowish supernatant; middle layer is an opaque fluid containing the PBMC; and bottom layer is RBC.
7. Quickly transfer the buffy coat layer of PBMC into a new 15ml centrifuge tube
8. Add 4ml of diluent buffer to the lymphocyte layer and mix by gentle pipetting.
9. Centrifuge at 1600rpm in a Swing Bucket Rotor (or 1800rpm in a Fixed Angle Rotor) for 10 minutes at 4°C.
10. Discard the sup and repeat the above step. This step helps to reduce the number of platelets.
11. Finally resuspend the pellet in 500µl of diluent buffer.

Staining Procedure

Fixation:

Streak a thin smear across a sterile slide by means of another sterile slide or cover slip. Air-dry quickly.

Staining:

1. Pour few drops (not more than 1ml) of Wright's solution over the smear and keep for 1-3 minutes.
2. Add around 2ml of water over the smear and let it stand twice as long as above step.
3. Rinse the smear with water until the edges show faint pinkish-red colour.
4. Blot dry carefully and check under microscope.

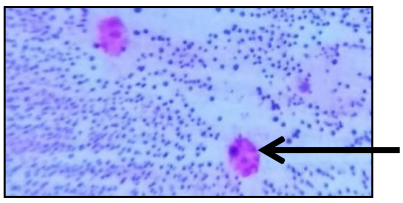

Fig: Lymphocyte under 100X magnification

Adapted from internet