Chronic lymphocytic leukemia (CLL) cell isolation

From: Duke/UNC/UTA/EBI ENCODE group
Date: 8/24/10

1) Source of cells: Dr. Jennifer Brown, Department of Medicine, Harvard Medical School.

2) Fresh peripheral blood drawn from CLL patients. Buffy coat was used to isolate lymphocytes, which are primarily B cells from these individuals.

3) Donor information: CLL patients, female and male

Lymphocyte separation protocol:

Modified from instructions for LSM Lymphocyte separation medium (50494) from MPBiomedicals Inc.

1. Thoroughly mix the LSM by inverting the bottle gently
2. Aseptically transfer 3 ml of LSM to a 15 ml centrifuge tube.
3. Mix 2 ml of blood with 2 ml of 1 x RPMI medium 1640 (Invitrogen, catalog # 21870)
4. Carefully layer the diluted blood over 3 ml of LSM in a 15 ml centrifuge tube, creating a sharp blood-LSM interface. Do not mix diluted blood into the LSM.
5. Centrifuge the tube at 400 x g at room temperature for 40 min. Centrifugation should sediment erythrocytes and polynuclear leukocytes and band mononuclear lymphocytes above LSM.
6. Aspirate the lymphocyte layer plus above half of the LSM layer below it and transfer it to a centrifuge tube. Add an equal volume of buffered balanced salt solution to the lymphocyte layer in the centrifuge tube and centrifuge for 10 min at room temperature at speed of 260 x g.
7. Wash the cells again with 10 ml of 1X PBS