

Ficoll gradient procedure for CLL-cells

1. Dilute the fresh blood 1:1 with PBS* at room temperature (RT). Caution: blood hazard !! Hepatitis B/ AIDS hazard!! Therefore be extremely careful with human blood !! Get Hepatitis B vaccination !! Try to wear double latex gloves. All soluble wastes have to be decontaminated. Use bleach or a different decontaminant.
2. Dispense 20 ml of Ficoll-Paque** in sterile 50 ml falcon tubes and overlay 1:1 with diluted blood (important: phases should not mix !!) to separate the lymphocytes and monocytes from the erythrocytes and blood-plasma.
3. Centrifuge in Sorvall table top centrifuge model: RT 6000B at RT at 1800 rpm for 25 min brake off (, because of ficoll- gradient !!).
5. Carefully take interphase out with a pipette and transfer it to a new 50 ml falcon tube. 6. Wash the lymphocytes 2 x with PBS at 1500 rpm, at RT, 10 min, with brake on in Sorvall table centrifuge. 7. Use CLL-P100- protocol to purify BCL2 containing membranes. *PBS: 25 x: 5g Kcl 5g KH₂PO₄ 29g Na₂HPO₄ x 2 H₂O 200g NaCl dilute to 1x with MQ water and autoclave before use. **Ficoll-Paque: purchase from Pharmacia LKB Code No: 17-0840-03