PROTOCOL 1.

PROTOCOL 1. ISOLATION of adult stem cells from BONE MARROW (Blood)

MATERIALS:

- 15ml tube with Ficoll (4ml)
- 15ml tube with blood (8 ml - 1:1 with 2mM EDTA/PBS)
- 1x 15ml tube
- Pasteur pipette - few

PROCEDURE

1. Dilute blood 1:1 with PBS contain 2mM EDTA
2. Add 4ml of Ficoll into the 15ml tube.
3. Transfer, VERY SLOWLY, drop by drop blood on the top of Ficoll. TO DO NOT MIX Ficoll with blood.
4. Spin for 30min at 1800g (2840 rpm) **break off** at 17°C

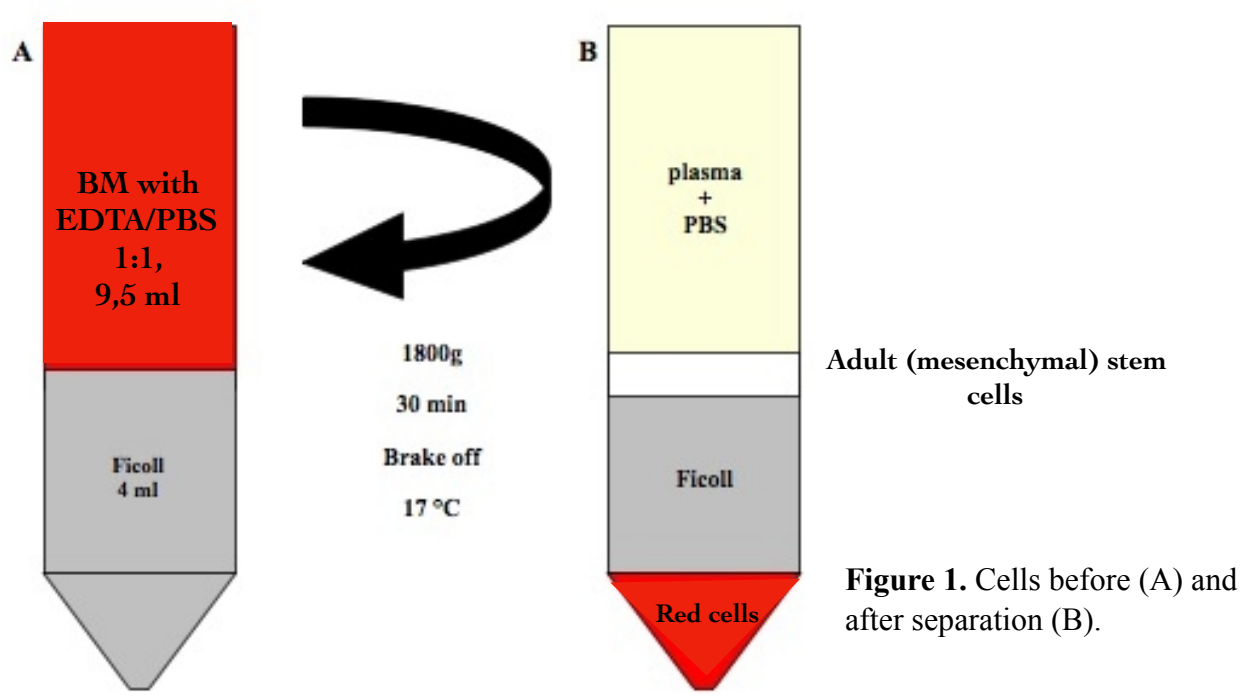

Figure 1. Cells before (A) and after separation (B).

5. After centrifuge - VERY CAREFULLY transfer “white level” containing lymphocytes (Bone marrow mononuclear cells) into new 15 ml tube. CAREFULLY TO DO NOT CONTAMINATE with other cells.
6. Add PBS up to 5ml.
7. Spin 5 min at 1200 rpm.
8. Trash supernatant and re-suspend cells in 1ml of MEM.
9. COUNT number of vital cells and freeze.