Preparation of Blood

Reagents:

- Fresh whole blood
- RPMI medium; [Gibco]
- Sepacell-R-500 (II) filtration unit; [Asahi Medical]

Protocol:

1. Filter the blood pack with the leukocyte reduction filter for red cells (Sepacell-R-500 (II)). Filtration must be done @ 4.0°C. Be sure to use sterile technique, i.e. connect the filtration unit under the sterile hood, etc.
2. Aliquot the filtered blood in 50mL tubes and centrifuge @ 1250xg for 5 min @ 4.0°C.
3. Transfer the supernatent (Plasma) into a new 50mL tube. Plasma must be prepared as human serum with the following modifications: After incubation @ 56°C, spin the plasma @ 3200xg for 15 min, and take the supernatent to filter with a Millipore filter.
4. Add 25mL incomplete RPMI (no serum or albumax) to 25mL blood and mix well.
5. Centrifuge @ 1250xg for 5 min (don't forget to turn off the brake)
6. Remove the supernatent and the Duffy coat, which will appear as a pale layer of cells at the interface (this contains left over white blood cells).
7. Wash and centrifuge again as in steps 4 & 5.
8. Remove the supernatent and any buffer coat that remains.
9. Resuspend washed cells in an equal volume of Complete RPMI medium and store @ 4.0°C (**Final 50% Haematocrit**). Blood prepared in this manner is adequate for up to three weeks.