

## **Rabbit Buffy Coat Cell Protocol-Modified Version**

**Mike Sun, 1/16/03**

1. Draw 3ml of rabbit peripheral blood into test tube containing 20 units of heparin.
2. Mix gently the above solution with 10ml PBS (AMP+?) culture media.
3. Centrifuge using IEC-HNSII at approx. 1000rpm (1/2 max setting) for 10min.
4. Discard all supernatant except for 200ul directly above the cell pellet. (note that the yellowish and white buffy-coat cells which is the whole mixture of white blood cells will be on top of the cell pellet)
5. Gently remove the buffy-coat layer with the remaining supernatant.
6. Centrifuge using a 1.5 ml tube in microcentrifuge (Marathon) for 5 min at setting #2.
7. Again discard supernatant and collect buffy coat cell layer carefully into 500ul of PBS solution.
8. Mix gently and repeat step 6 and 7.
9. The buffy coat cell suspension in PBS is now ready for cell count and adenovirus infections. The total cell number obtained from 3ml of blood is approx.  $2 \times 10^7$ .