B-CELL PREPARATION

1. Draw blood into heparinized syringes.

3. Lyse RBCs if use cord blood.

4. Add 15cc Ficoll to 50cc polypropylene tubes (avoid cells sticking to tube walls). Layer 15-30cc whole blood onto Ficoll.

4a. When layering whole blood and Ficoll together, can either put blood in first and then gently layer Ficoll into bottom (place pipette through blood) or put Ficoll in first and then carefully layer blood on top.

5. Spin 1800 rpm x 35 min. at room temp. in 50 cc tubes.

6. Remove buffy coat (on top of Ficoll) with 5 cc pipette (can take a little Ficoll if necessary -usually take ~5-7cc volume/buffy coat).

7. Place 3 buffy coats into a 50cc tube (no more than 25cc total or cells may not pellet if too much Ficoll present).

8. Wash with 1x sterile PBS (fill tube to 50cc) and spin 1500rpm (~3.5) x 10 min at room temp.

9. Remove supernatant. (Leave ~5cc if some cells stuck to angled walls at bottom of tube.)

10. Resuspend cells (may clump at this point). Wash again with 1x sterile PBS (add ~35cc) and spin 800-1000rpm x 10 min. (slower speed helps to get rid of platelets).

11. Remove supernatant. (Again leave ~5cc if some cells stuck to angled walls at bottom of tube.)

11a. Pool cells obtained from ~100cc of blood into a 50cc tube.

12. Add sterile PBS to ~40cc total volume. Pipette up and down with a 5 cc pipette to resuspend and to break up clumps.

13. Count cells with 40x objective (should get ~1-2.5 million PBLs/cc blood). Dilute cells 1:5 (20ul cells : 80ul trypan blue) to count cells.

For EBV-negative blood donor proceed with steps 14. - 16:

14. Remove aliquots of 15-30 million PBL's (for 1-2 plates) and put into a 50cc tube (or a 15cc tube if volume small enough). (Want 150-200,000 PBL's/well at 96 wells/plate or 15-20 million cells/plate.)

15. Spin 1200rpm (control knob ~2.8) x 5 min. at room temp.

16. Remove supernatant with pasteur pipette-cells are ready to be infected..

For EBV-positive donor:

17. T-cells need to be rosetted out (see rosetting protocol). After rosetting, aliquot 5-10 million cells into tubes (for 1-2 plates). (Want ~50,000 rosetted cells /well at 96 wells /plate or 5 million cells/plate.) Then see steps 15. and 16.