USDA-ARS Beltsville Lunney Lab

Standard Operating Procedure for US-VIRN

Protocol title: Pig Peripheral Blood Mononuclear Cell (PBMC) Preparation

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1. Collect fresh blood into 10 ml vacutainer with anti-coagulant or into a 50ml tube with 1.2 ml of 0.2 M EDTA if from fresh kill.
2. *Pour 15-20 ml of blood into 50 ml tubes and bring to 35 ml by adding PBL Prep medium. Mix and underlay 7 ml of LSM (Lymphocyte Separation Medium, MP Biomedical, #50494) slowly in the bottom of the tube. It should form a clean interface.
3. Centrifuge at 2000 rpm (1200 G) for 30 min at room temperature, no brake.
4. Take the top white "fuzzy" cell band and put into a 15 ml tube. Fill tube with PBL Prep medium to 15 ml
5. Centrifuge 1000 rpm (300 G)for 10 min, low brake at room temperature.
6. Pour out supernatant and break the pellet with ratcheting motion (Best done over 15ml conical rack). Fill tube with PBL Prep medium to 15 ml = 2nd wash.
7. Centrifuge 1000 rpm (300 G)for 10 min, normal brake at room temperature.
8. For 3rd wash repeat as for #6 but fill tube with RPMI-1640 (Ca containg medium). NOTE: If there are red cells, add 1.5 ml distilled water and pipette up and down rapidly for 10 seconds before adding RPMI.
9. Centrifuge 1000 rpm for 10 min, normal brake at room temperature.
10. Pour out supernatant, break the pellet and resuspend with 1-4 ml of appropriate medium depending on cell pellet size. We use blastogenic medium for most PBMC cultures. Check for clumps. If any clumping filter immediately through a mesh cell filter.
11. Count number of cells and viability: Take 10µl aliquot into a small tube and dilute (1:20) with Trypan Blue. (Large pellet use 1:40 dilution). Count cells using a hemocytometer. Calculate cell concentration: # cells/ml = cell count x 20 (dilution used) x 10,000
    Viability % = (# live cells / # live and dead cells) x 100

2.*Note: If using only 1 vaccutainer (~10ml of blood) pour 5-6mls blood into a 15ml conical and fill with PBL up to 12mls and invert several times. Underlay with a 9inch Pasteur (~3mls) of LSM medium. Follow remaining procedure.

Solutions:

PBL Prep Medium

50ml 10X HBSS w/o Ca or Mg
25ml 0.2M EDTA
5ml 1M HEPES pH 7.3
420ml sterile H2O
Final Volume 500ml

Blastogenic Medium

10ml 200mM L-glutamine (100x)
10ml Pen/Strep (100x)
1ml 2-ME (1000x)
100ml fetal bovine serum (FBS)
10ml 1M HEPES pH 7.3 (100x) final 0.01M
870ml RPMI 1640
Final Volume 1L
Final pH: 7.0 – 7.2