Lymphocytes purification from cattle and caprine blood samples - May 28th 2015

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REAGENTS/ SOLUTIONS:
PBS/EDTA (= PBS without Ca and Mg (lab made) with EDTA 2 mM)(ref Sigma E5134)
Histopaque-1077 (Sigma-Aldrich ref 10771)
PBS/EDTA/FCS(= PBS with EDTA 2 mM +5% FCS + (optional) 1% P-S (= Penicillin-Streptomycin)
Lysis buffer (= NH_4Cl 155mM + $KHCO_3$ 10mM + EDTA 0.2 mM)
RPMI 1640 with L-glutamine and $NaHCO_3$ ref Sigma-Aldrich R8758
RPMI/FCS (with 10% FCS)
RPMI/DMSO (= RPMI with 10% DMSO ref Sigma D2650)

EQUIPMENT:
50ml polypropylene tubes
Centrifuge
1.8 ml cryotubes
Cell freezing container CoolCell®LX (BioCision)

PROCESS

1. **Dilution of the blood:**

   Prepare 50ml polypropylene tubes containing 25 ml PBS EDTA/ 2mM buffer. Dilute the collected blood **at least to half** by pouring 25 ml blood into the buffer. Homogenize gently.

2. **Purification of Mononuclear cells**:

   a. Distribute gently 30-35 mL of diluted blood over 15ml Histopaque-1077 layer without mixing the two layers

   b. Spin at 1500g for 30', at room temperature (set the lowest acceleration and deceleration of the centrifuge).

   c. Transfer the PBMCs layer into a 50ml polypropylene tube filled with 10 ml of PBS/EDTA/FCS. Fill tube up to 50ml with PBS/EDTA/FCS medium.

   d. Spin at 450/500g for 10', at 4°C

   e. Resuspend cells in PBS/EDTA/FCS

   f. Spin at 400g for 10', at 4°C

3. **Red blood cell lysis:**

   a. Add 7 ml of lysis buffer on each pellet. After 2 minutes, stop the lysis by adding RPMI10%FCS medium up to 45 ml (this step is to remove red blood cells)

   b. Spin at 400g for 10', at 4°C

c. Resuspend the cell pellet in 10 ml RPMI/10%FCS medium and count cells

b. Spin at 400 g for 10', at 4°C.

4. Cell freezing:

a. Resuspend 10^8 PBMCs in 1.8 ml of cold FCS /DMSO in cryotubes (work on ice).

b. Transfer cryotubes to a cell freezing container and place at -80°C

c. About 48 to 72 hours later, transfer cryotubes to a liquid nitrogen container for long term storage.