Isolating PolyMorphoNuclear Cells (PMNs) Protocol

1. Draw blood from a donor in the heparin coated tubes
2. Transfer the blood into a 50 ml conical tube and add the same amount of pre-warmed RPMI
3. In another 50 ml conical tube add 15 ml Ficoll. By pipetting up and down homogenize the blood and the media in the tube from step 4 and slowly lay the blood over Ficoll (35 ml blood max over 15ml Ficoll).
4. Centrifuge for 30min at 1400 rpm, R.T.
5. Take of as much of the ficoll and other top layers without disturbing the pellet.
6. Add the same amount of RPMI (at 4 C) as the red blood cell layer and mix up and down gently.
7. Add 50% of the total volume of pyrogen-free Dextran (at 4C) to the tube and leave in the cold room or fridge for 1hr 15'.
8. Remove from fridge and pipette top layer to a new 50 ml conical tube, being careful not to disturb the RBC layer.
9. Fill the tube with RPMI to the top and spin the supernatant to pellet the PMNs, 700rpm for 6min. Pour off supernatant as waste.

10. Use 2-5ml lysis buffer to lyse RBCs. Incubate at r.t. in the dark for 2 min (Not Longer). *Lysis buffer is stored at 4C and is 10X. Before using, make up 5ml 1X buffer in distilled water.
11. Bring cells up in 50 ml RPMI and spin to pellet, 700rpm for 6 min. Pour off supernatant as waste.
12. Bring up in preferred media and count PMNs.