ImageStream® CD45/Syto13 human whole blood cell classification

Experimental Procedures

Samples

Single fluorescent color control samples – use 200 µl for single color control. You can mix non-Syto single color controls.

Experimental samples – use 500 µl and stain according to following protocol.

Materials

01. anti-CD45 PerCP : BD (Cat. 347464), 5X
02. anti-CD14 PE: Caltag
03. anti-CD16 PE: Caltag
04. Syto-13: Molecular Probes (Cat.)
05. BD FACS-lyse
06. Phosphate buffered saline without Ca^{2+}/Mg^{2+} (PBS)
07. Lavender Top Vacutainer
08. Holder
09. Needle

Cell preparation

Draw blood in anticoagulant such as heparanized (green top) or K3 EDTA (lavender top) vacutainer tubes. Assume 5-10 x10^6 cells per ml. Tubes should be processed immediately, as CD45 levels will change at RT. Aliquot appropriate amount to 15 cc conical centrifuge tubes. Place on ice prior to staining.

01. Stain CD45 PerCP (1:5) and CD16PE (1:30) or CD14PE (1:20) 10' on ice
02. Add 10X volume of 1X BD FACS-lyse at RT for 10' to lyse RBC and fix cells
03. Centrifuge 300xg 10'
04. Resuspend pellet in PBS at 10^6 cells/ml and filter 70 um mesh
05. Centrifuge 300xg rpm 10'
06. Resuspend 5-10 x10^7 cells per ml in PBS + 200 nM Syto13 and run on ImageStream