

ImageStream® CD45/DRAQ5 Human Whole Blood Cell Classification

Experimental Procedures

Samples

Single fluorescent color control samples – use 200 µl whole blood for single color control.

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

Materials

01. anti-CD45 FITC: Caltag (Cat. MHCD4501)
02. DRAQ5: Biostatus (Cat. DR50050; 5Mm stock)
03. BD FACS-lyse (Cat. 349202; 10X stock)
04. Phosphate buffered saline without $\text{Ca}^{2+}/\text{Mg}^{2+}$ (PBS) + 2% FBS (wash buffer)
05. Lavender Top Vacutainer
06. Holder
07. Needle

Cell preparation

Draw blood in anticoagulant such as heparanized (green top) or K3 EDTA (lavender top) vacutainer tubes. Assume $5\text{-}10 \times 10^6$ 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 0.5 mL blood with CD45 FITC (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 5 mL wash buffer
05. Centrifuge 300xg 10'
06. Resuspend 50 µL in wash buffer + 20 µM DRAQ5 and run on ImageStream

Instrument setup

Collect with 200 mW 488 laser power, BF in channel 5. CD45 FITC will be in Ch3, DRAQ5 in Ch6. While looking at Ch3 intensity (log) vs Ch1 intensity plot, adjust laser height using scatter controller to maximize discrimination of 5 cell types in whole blood. Eosinophils will scatter the most and have intermediate CD45 intensity, while lymphocytes will scatter the least and have high CD45 intensity.