ImageStream® Nuclear Translocation Protocol – whole blood

Probing Nuclear Translocation of NF-κB in whole blood samples using the ImageStreamX

Samples: (5 x 10^5 cells per test)

Single fluorescent color control samples – unstained, NFκB FITC, DRAQ5

Experimental samples – untreated, positive control treatment, experimental treatment

Materials

1. Lavender Top Vacutainer, holder and needle
2. rabbit anti-NFκB (p65) : Santa Cruz Biotechnology (Cat. SC-372), 200 μg/ml
3. FITC F(ab')$_2$ donkey anti-rabbit IgG (H+L): Jackson Immunoresearch (Cat. 711-096-152), 1.5 mg/ml
4. DRAQ5: Axxora (Cat. BOS 889 001 R200), 5mM stock
5. Phosphate buffered saline without Ca^{2+}/Mg^{2+} (PBS)
6. Fix-FACS-lyse (6:3:1 mix of diH20, 10% formaldehyde (Polysciences # 04018), FACS-lyse (BD #349202)
7. 0.1% triton X-100/2% FBS/0.1% azide/PBS (PermWash Buffer)
8. 2% FBS/PBS (Wash buffer)
9. Siliconized polypropylene tubes: Sigma (Cat. T4691 for 0.6mL and Cat. T4816 for 1.5mL)

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. Aliquot appropriate amount to 15 cc polypropylene conical centrifuge tubes or siliconized polypropylene (NOT POLYSTYRENE) microcentrifuge tubes. All washes done at 300 x g 10' 4°C in a swinging bucket rotor

1. Stimulate blood at 37°C under 5% CO_2 humidified atmosphere
2. Stain for surface receptors 10' on ice (or after fix)
3. Add 10X volume Fix-FACS-lyse at RT for 10' to lyse RBC and fix cells
4. Centrifuge 300xg 10'
5. Wash with Wash buffer, resuspend in 100 μL PermWash Buffer containing 1:20 (10 μg/mL) anti-NFκB 20' 25°C.
6. Wash with PermWash buffer, resuspend in 100 μL PermWash buffer + FITC F(ab')$_2$ donkey anti-rabbit IgG (1:200 = 7.5 μg/mL) 15' 25°C.
7. Wash with Wash buffer, resuspend 50 μL 1%PFA (or Wash buffer if run on same day) + 50 μM DRAQ5 5' (1:100) and run directly on ImageStream in 0.6 mL microcentrifuge tubes.