## Probing Nuclear Translocation of NF-κB

### Samples: (3 x $10^6$ cells per test)

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

Experimental samples – untreated, positive control treatment, experimental treatment

### Materials

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

### Cell preparation

We used THP-1 cells cultured in RPMI supplemented with 5% fetal calf serum in an incubator containing 5% $CO_2$ at 37° C. THP-1 cells (at a density of at most 5E+05 cells/mL) were stimulated with or without LPS to induce nuclear translocation of NF-κB.

### Staining protocol

Staining done in siliconized polypropylene (NOT POLYSTYRENE) microcentrifuge tubes

All washes done at 300 x g 10' 4°C in a swinging bucket rotor

1. Culture cells to mid-exponential growth in culture flasks containing 5% FCS/ RPMI.
2. Stimulate the cells for 37°C under 5% $CO_2$ humidified atmosphere.
3. Wash and stain surface proteins on ice in Wash buffer.
4. Wash with Wash buffer and fix cells in 100 μL Fixation Buffer 10' 25°C.
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.