Nuclear Translocation Protocol

Probing Nuclear Translocation of NF-κB

Samples: (3 x 10^6 cells per test)
- Single fluorescent color control samples – unstained, NFκB FITC, 7-AAD
- Experimental samples – untreated, positive control treatment, experimental treatment

Materials
01. rabbit anti-NFκB (p65) : Santa Cruz Biotechnology (Cat. SC-372), 200 µg/ml
02. FITC F(ab’)_2 donkey anti-rabbit IgG (H+L): Jackson Immunoresearch (Cat. 711-096-152), 1.5 mg/ml
03. 7-AAD: Molecular Probes (Cat. A-1310)
04. Phosphate buffered saline without Ca²⁺/Mg²⁺ (PBS)
05. 4% PFA/PBS (Fixation Buffer)
06. 0.1% triton X-100/3% FBS/0.1% azide/PBS (PermWash Buffer)
07. 2% FBS/PBS (Wash buffer)
08. Siliconized polypropylene tubes

Cell preparation
We used THP-1 cells cultured in RPMI supplemented with 5% fetal calf serum in an incubator containing 5% CO₂ at 37°C. THP-1 cells 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

01. Culture cells to mid-exponential growth in culture flasks containing 5% FCS/ RPMI.
02. Stimulate the cells for 37°C under 5% CO₂ humidified atmosphere.
03. Wash and stain surface proteins on ice in Wash buffer.
04. Wash with Wash buffer and fix cells in 100 µL Fixation Buffer 10’ 25°C.
05. Wash with Wash buffer, resuspend in 100 µL PermWash Buffer containing 1:20 (10 µg/mL) anti-NFκB 20’ 25°C.
06. Wash with PermWash buffer, resuspend in 100 µL PermWash buffer + FTTC F(ab’)₂ donkey anti-rabbit IgG (1:200 = 7.5 µg/mL) 15’ 25°C.
07. Wash with Wash buffer, resuspend 50-100 µL 1%PFA (or Wash buffer if run on same day) + 40 µM 7-AAD 5’ and run directly on ImageStream.