
INTRACELLULAR STAINING OF HUMAN NF-KB IN ADHERENT CELLS

MATERIALS

1. Adherent cells
2. TBS
3. Fixative- 3.7% wt/v paraformaldehyde in TBS
4. Permeabilisation buffer- 0.2% Triton-X100 in TBS
5. Blocking buffer- 10% serum normal goat serum) in TBS
6. 1° Antibody: Rabbit anti-NF- κ B (Santa Cruz sc-372) used at 1/50 in blocking buffer
7. 2° Antibody: Goat anti-Rabbit AF-633 (Invitrogen A21072) used at 1/500 in blocking buffer
8. DAPI (2 μ g/ml) in TBS
9. Mounting solution (Vectashield hard set)

INTRACELLULAR STAINING OF HUMAN NF-KB IN ADHERENT CELLS

METHODS

1. Aspirate media & fix cells for 15 minutes in 3.7% paraformaldehyde
2. Wash cells x3 with TBS
3. Permeabilise cells with **0.2%** Triton-X100 for **10 minutes** at room temperature & wash x3 with TBS
4. Incubate with blocking buffer for 30 minutes at room temperature
5. Remove blocking buffer and incubate with 1° antibody (in blocking buffer) **overnight at 4°C**
6. Remove 1° Ab & wash x3 with TBS
7. Incubate with 2° reagent for 1h at room temperature
8. Remove 2° reagent & wash x3 with TBS
9. Incubate with DAPI (2 µg/ml in TBS) for 5 minutes
10. Wash DAPI x3 with TBS
11. Mount
12. Store @ 4°C in the dark