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Quick Reference Card

Amnis® NFκB Translocation Kit
ACS10000

Two-color assay kit for rapid detection and quantitation of NFκB translocation from the cytoplasm to the nucleus

For Research Use Only. Not for use in diagnostic procedures.

Storage Conditions

Store the Anti-Hu NFκB Alexa Fluor® 488, 7-AAD Solution, 5X Assay Buffer, and Permeabilization Buffer at 2–8 °C. Store the 5X Fixation Solution at room temperature.

Kit Components

- Anti-Hu NFκB (p50) Alexa Fluor® 488 clone 4D1
  (Part No. 4700-1674, 50 tests/vial)
- 7-AAD Reagent
  (Part No. 4000-0290, 50 tests/vial)
- 5X Fixation Solution
  (Part No. 4300-0340, 2 x 3 mL vials)
- 5X Assay Buffer
  (Part No. CS202124, 50 mL/vial)
- Permeabilization Buffer
  (Part No. CS202125, 13 mL/vial)

Materials Recommended

- ImageStream® or FlowSight® imaging cytometer
- Cells of interest (1 x 10^6 cells/test)
- NFκB stimulation reagent
- Tissue culture instruments and supplies
- Siliconized polypropylene (low retention) tubes
- Micropipettors
- Disposable micropipettor tips
- Tabletop centrifuge capable of exceeding 300 x g
- Vortex mixer
- 1X Phosphate-buffered saline (PBS) without calcium or magnesium
- Deionized water

See reverse for Assay Protocol

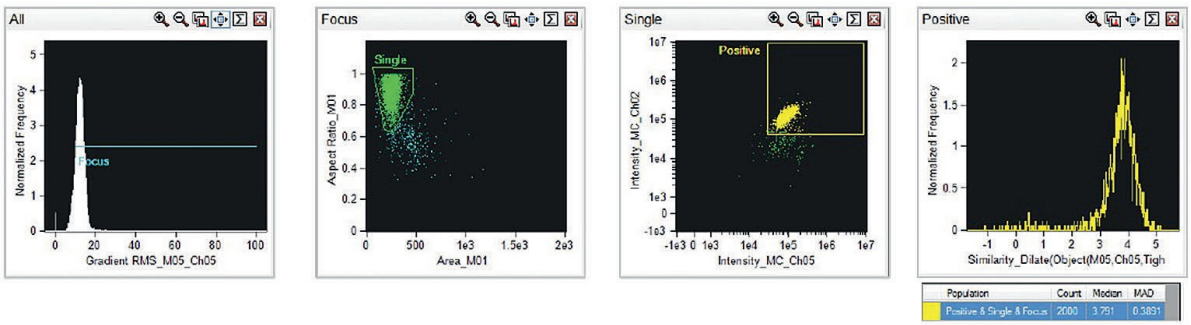

Figure 1. Sequence of Analysis Steps in Evaluating NFκB Translocation: A. Gate cells in best focus. B. Gate on single cells. C. Gate double positives. D. Gate translocated events. The Similarity histogram represents how similar the NFκB image is to the 7-AAD image.

Assay Protocol

This protocol was developed using 10^6 cells per test.

Prepare stimulated and control cells, including positive, negative and single-color controls

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Centrifuge cells and wash with PBS, transfer cells with 1 x 10^6 cells per test into each tube

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Prepare working solutions of 1X Fixation Buffer (20 µL 5X Fixation Solution and 80 µL 1X PBS per test), 0.25X Fixation Buffer (5 µL 5X Fixation Solution and 95 µL 1X PBS per test) and 1X Assay Buffer (0.4 mL 5X Assay Buffer and 1.6 mL dH_2O per test)

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Optional: Stain with surface markers (follow the surface markers protocols)

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Optional: Wash with PBS or 1X Assay buffer to remove any excess surface marker probe and pellet cell

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Fix the cells with 100 µL 1X Fixation Buffer for 10 min at room temperature

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Wash and pellet with 1X Assay Buffer

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Prepare working solution of Anti-Hu NFκB Alexa Fluor® 488 Antibody/ Permeabilization Buffer by adding 5 µL anti-Hu NFκB Alexa Fluor® 488 antibody to 95 µL Permeabilization Buffer per test

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Add 100 µL of the Anti-Hu NFκB Alexa Fluor® 488 Antibody/ Permeabilization Buffer to each test, mix thoroughly and incubate for 30 min at room temperature

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Wash and pellet with 1X Assay Buffer

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Resuspend cells in 100 µL 0.25X Fixation Buffer

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Add 10 µL 7-AAD Reagent per test and incubate 5 min

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Run samples (including single-color controls) on the ImageStream® or FlowSight® cytometer

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Analyze data files with IDEAS® software using the Nuclear Localization wizard

A detailed user's guide can be found at *www.cytekbio.com*.

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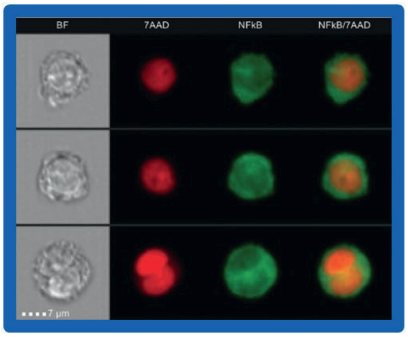
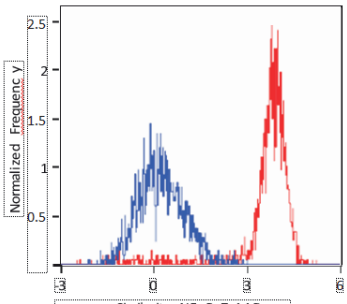
Example Data

Median Similarity Control Cells





Median Similarity Score
Control (no LPS) = 0.2
1µg/mL LPS treated = 3.8

Median Similarity LPS Treated Cells

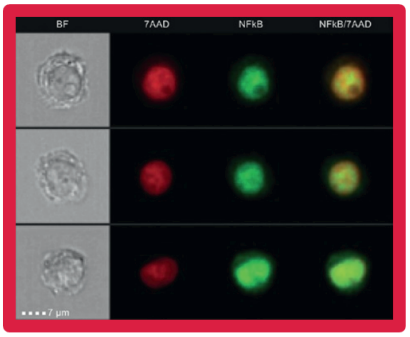


Figure 2. Example ImageStream data. Histograms for the control (blue) and the 1 µg/mL LPS-treated (red) THP-1 cells are shown, along with representative 60X images of the selected regions. The brightfield image, Anti-Hu NFκB Alexa Fluor® 488 (green), 7-AAD (red) and a composite of the Anti-Hu NFκB Alexa Fluor® 488 and 7-AAD images are shown.