

Thermo Scientific NF κ B p65 Redistribution[®] Assay (U2OS Cells)

The Redistribution technology monitors the cellular translocation of GFP-tagged proteins in response to drug compounds or other stimuli and allows easy acquisition of multiple readouts from the same cell in a single assay run. In addition to the primary readout, high content assays provide supplementary information about cell morphology, compound fluorescence, and cellular toxicity.

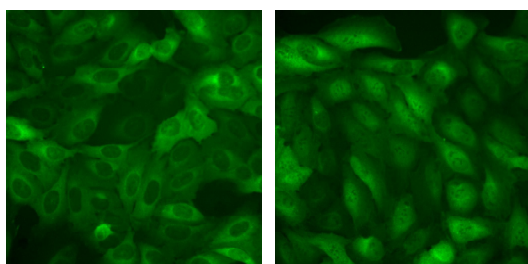


Figure 1. Translocation of NF κ B-EGFP in response to TNF α . Cells were treated with 0.25% DMSO (control, left panel) or 10 ng/ml TNF α for 30 min (right panel). The cytoplasm to nucleus translocation is detected by the image analysis algorithm.

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Nuclear factor- κ B (NF κ B) is a nuclear transcription factor which regulates the expression of a large number of genes critical for several processes, including apoptosis, viral replication, tumorigenesis, inflammation, and various autoimmune diseases. Activation of NF κ B is part of a stress response, and it is activated by growth factors, cytokines, lymphokines, UV light, pharmacological agents, and stress. Five mammalian NF- κ B family members are identified (p50, p52, p65, RelB and c-Rel). The transcription factor NF- κ B works only when two members form a dimer. The most abundant form consists of a p50 or p52 subunit and a p65 subunit. In its inactive form, NF κ B is located in the cytoplasm, bound by members of the I κ B family of inhibitor proteins. Stimuli such as interleukin-1 β or TNF α cause phosphorylation of I κ B, which leads to its ubiquitination and subsequent degradation of the inhibitor protein. This results in nuclear translocation of NF κ B and increased NF κ B-mediated gene expression. NF κ B

nuclear translocation can be inhibited by the I κ B α specific inhibitor RO 106-9920, which inhibits ubiquitination and degradation of I κ B α and subsequent nuclear import of NF κ B [1,2].

Features

- Designed to assay compounds for their ability to modulate nuclear translocation of NF κ B
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications

Highlights:

- **Biologically relevant data**
Compounds tested in a cellular environment
- **Validated**
Functionally tested cells provided with an optimized assay protocol
- **Easy to use**
Just plate cells, add compounds, and image

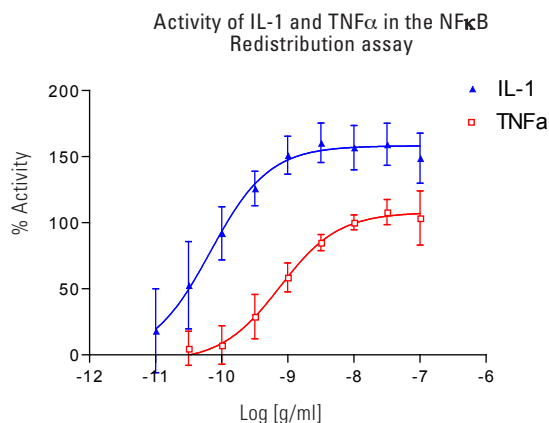


Figure 2. IL-1 and TNF α concentration response curves in the NF κ B Redistribution assay. Concentration response was measured in 9 point half log dilution series of IL-1 or TNF α . Cells were incubated with test samples for 30 min. Cells were then fixed and the nucleus to cytoplasm translocation was measured using the Cellomics ArrayScan V^{TI} Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (10 ng/ml TNF α) and negative control (0.25% DMSO). The EC₅₀ of IL-1 is ~0.07 ng/ml and the EC₅₀ of TNF α is ~0.7 ng/ml.

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Assay Details

Recombinant U2OS cells stably expressing human NF κ B fused to the N-terminus of enhanced green fluorescent protein (EGFP). The NF κ B Redistribution assay is designed to measure NF κ B activation by monitoring the translocation of the NF κ B p65-EGFP fusion protein from the cytoplasm to the nucleus. The NF κ B cell line is responsive to both interleukin 1 (IL-1) and to tumor necrosis factor alpha (TNF α). The assay has been optimized and validated in agonist format using TNF α as agonist. The NF κ B assay is validated with an average Z' = 0.50 \pm 0.12, suitable for both screening and profiling applications.

Imaging

The translocation of NF κ B p65-GFP can be imaged on most HCS platforms and fluorescence microscopes. The filters should be set for Hoechst (350/461 nm) and GFP/FITC (488/509 nm) (wavelength for excitation and emission maxima). Consult the instrument manual for the correct filter settings. The translocation can typically be analyzed on images taken with a 10x objective or higher magnification. The primary output in the NF κ B Redistribution assay is the translocation of NF κ B p65-GFP from the cytoplasm to the nucleus. The data analysis should therefore report an output relating to the GFP fluorescence intensities in the nucleus and the cytoplasm.

Imaging on Thermo Scientific Cellomics ArrayScan V^{TI}

This assay has been validated on the Cellomics Arrayscan V^{TI} using a 10x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the Redistribution V3 BioApplication. The output used was MEAN_CircRingAvgIntenRatioLog (Log of the ratio of average fluorescence intensities of nucleus and cytoplasm (well average)). The minimally acceptable number of cells used for image analysis in each well was set to 700 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.

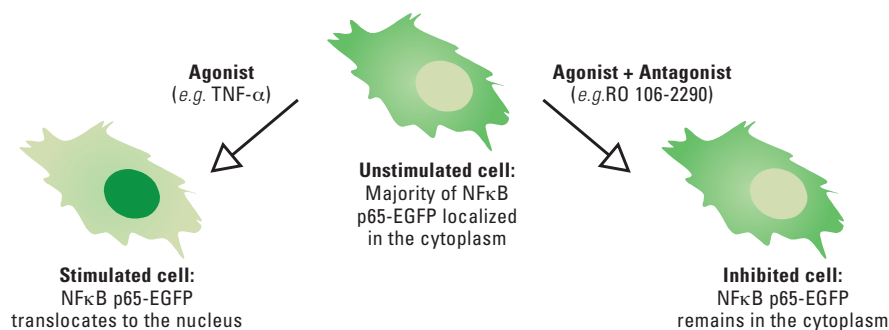


Figure 3. Illustration of the NFκB translocation event.

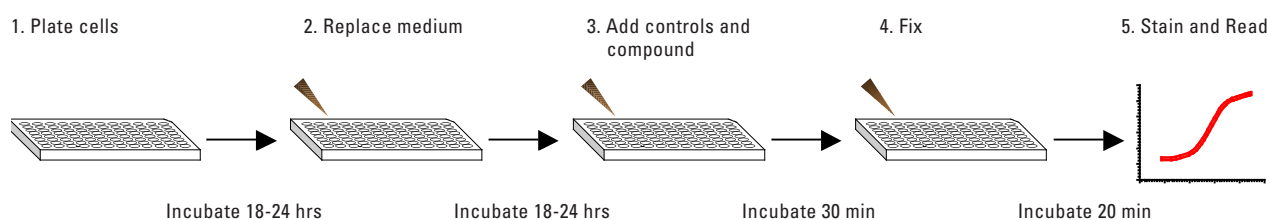


Figure 4. The NFκB Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
091_01	NFκB Redistribution Assay	U2OS	•	•	

The Redistribution Assays are available in 3 product formats, Profiling, Screening and CryoRedi, for different volume and level of convenience needs. The Redistribution Assays can also be accessed through the Thermo Scientific Managed Services.

Related Thermo Scientific Products

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
002_01	MK2 Redistribution Assay	CHO	•		
037_01	MK2 Redistribution Assay	U2OS	•	•	•
038_01	MK2EE Redistribution Assay	U2OS	•	•	
8400301	Cellomics NFκB & c-Jun Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8407901	Cellomics Phospho-c-Jun and Phospho-JNK Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100071	Cellomics ERK MAPK Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8402201	Cellomics Caspase 3 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
CX03004-INS	Cellomics ONE BioApplication Suite	High content data acquisition and analysis software			
CX03102A/B	Cellomics ArrayScan V ^{TI}	Flexible, high throughput, high content reader			
N01-3001	CelliWoRx	Economical high content reader			

References

1. Swinney DC et al., *J Biol Chem* 277, 23573-23581, 2002.
2. Schmid JA et al., *J Biol Chem* 275, 17035-17042, 2000.

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