Product Specifications

Thermo Scientific NFATc1 Redistribution® Assay

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.

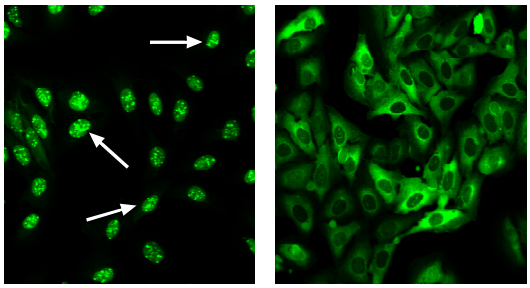

Figure 1. Images of cells stably transfected with EGFP-NFATc1. Cells have been treated with 1 µM ionomycin in the absence (DMSO control, left panel) or presence (right panel) of 300 nM FK506 antagonist. Antagonist treatment leads to nucleus to cytoplasm translocation of EGFP-NFATc1.

Thermo Scientific NFATc1 Redistribution Assay

Nuclear factor of activated T-cells c1 (NFATc1) is a transcription factor involved in T-cell signaling and tissue development, and its activity is controlled by the Ca^{2+}/Calmodulin-dependent phosphatase, calcineurin. Inactive NFATc1 transcription factor resides in the cytosol. In response to sustained elevated calcium levels, NFATc1 is dephosphorylated by calcineurin, which induces its rapid translocation to the nucleus. In the nucleus it forms transcription complexes with other transcription factors such as AP-1, GATA4, GATA2, and MEF2 [1,2]. NFATc1 dephosphorylation and nuclear translocation can be inhibited pharmacologically by the microbial products FK506 and cyclosporin A (CsA) [3]. These reagents bind to the intracellular proteins FKBPK and cyclophilin respectively, and they subsequently bind to calcineurin and block phosphatase activity.

Features

- Designed to assay compounds for their ability to modulate nuclear translocation of NFATc1
- 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

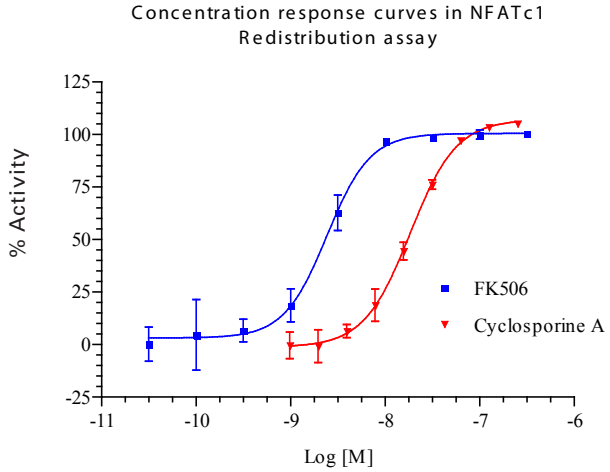

Figure 2. Concentration response curves in the NFATc1 Redistribution assay. Concentration response was measured in 9 point dilution series of cyclosporine A and FK506 (n = 4). Cells were incubated with test compound for 65 min before treatment with 1 µM ionomycin for 5 min. Cells were then fixed and the nucleus to cytoplasm translocation was measured using image analysis. % activity was calculated relative to the positive (300 nM FK506) and negative control (0.25% DMSO). The EC_{50} values are: FK506 EC_{50} = 2.4 nM, Cyclosporine A EC_{50}=18 nM.

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

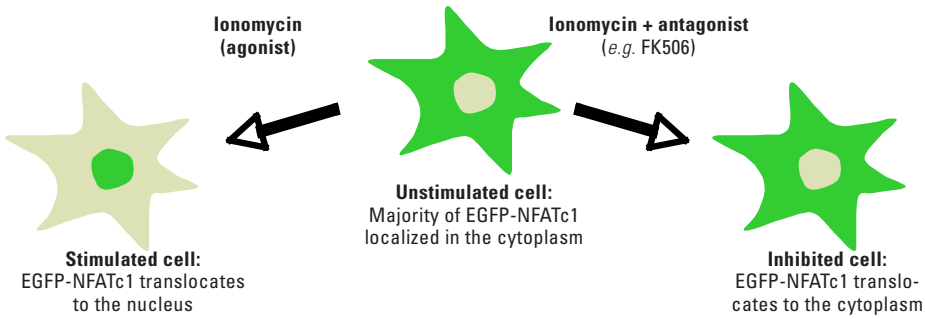

Figure 3. Illustration of the NFATc translocation event.

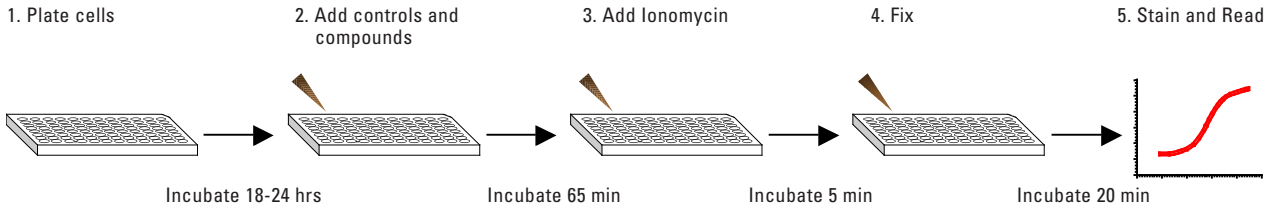

Figure 4. The NFATc1 Redistribution assay is very easy and fast to perform.

Thermo Scientific NFATc1 Redistribution® Assay

Assay Details

Recombinant U2OS cells stably expressing human NFATc1 fused to the C-terminus of enhanced green fluorescent protein (EGFP). The NFATc1 antagonist Redistribution assay is designed to screen for antagonists of NFATc1 translocation by monitoring the translocation of an EGFP-NFATc1 fusion protein from the cytoplasm to the nucleus. Ionomycin is used as the reference agonist compound and test compounds are assayed for their ability to inhibit ionomycin-induced cytoplasm-to-nucleus translocation of NFATc1. In this assay, FK 506 is used as a reference antagonist. Compounds inhibiting ionomycin-induced translocation could act by interfering directly with NFATc1 translocation, acting upstream of NFATc1 but downstream of the calcium signal, or they could be general inhibitors of nuclear import. The NFATc1 assay is validated with an average Z′ = 0.72 ± 0.10, suitable for both screening and profiling applications.

Imaging

The translocation of NFATc1 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 NFATc1 Redistribution assay is the translocation of NFATc1 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 developed 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 200 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2 and ColocalizationV3.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
017_01	NFATc1 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
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U2OS	•		
048_01	NK1_NFATc1 activation Redistribution Assay	U2OS	•	•	
073_01	M3-NFATc1 activation Redistribution Assay	U2OS	•	•	
078_01	AT1R-NFATc1 activation Redistribution Assay	U2OS	•	•	
079_01	MCHR1-NFATc1 activation Redistribution Assay	U2OS	•	•	
088_01	M1-NFATc1 activation Redistribution Assay	U2OS	•	•	
K0100111	Cellomics NFAT-1 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100041	Cellomics p38 MAPK Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8401201	Cellomics BrdU Cell Proliferation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8404601	Cellomics Cell Cycle I 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	CellWoRx	Economical high content reader			

References

1. Rao A et al. *Annu Rev Immunol.* 15, 707-747, 1997.
2. Masuda et al. *Cell Signal.* 10, 599-611, 1998.
3. Martinez-Martinez S & Redono JM. *Curr Med Chem.* 2004, 11, 997-1007, 2004.

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