

Thermo Scientific M3: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.

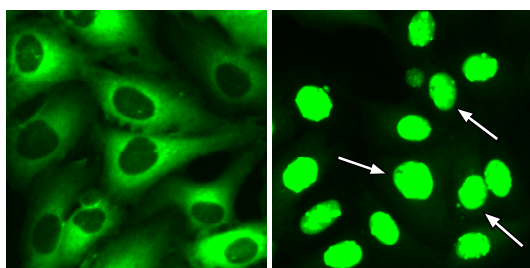


Figure 1. Nuclear translocation of EGFP-NFATc1. Cells expressing the M3 receptor were treated with 300 nM carbachol for 20 min (right panel) or untreated (DMSO control, left panel). Activation of the receptor leads to nuclear translocation of EGFP-NFATc1, which can be detected by the image analysis algorithm.

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The muscarinic acetylcholine receptor 3 belongs to a family of G protein-coupled receptors (GPCRs), which consists of five members (M1-M5). The odd-numbered members (M1, M3, M5) couple to $G\alpha_q$ whereas the even-numbered members (M2, M4) couple to $G\alpha_i$. Muscarinic acetylcholine receptors mediate the effect of acetylcholine in synapses, and they are involved in several indications related to the central nervous system [1]. In this assay, the muscarinic acetylcholine receptor 3 (M3) has been transfected into the NFATc1 GPCR Reporter Assay for Gq-coupled Receptors (Product ID 017_02), where receptor activation leads to release of cytoplasmic Ca^{2+} , which in turn induces NFATc1 translocation. Binding of an agonist to the extracellular parts of M3 causes a conformational change in the receptor. This leads to activation of heterotrimeric Gq proteins, subsequent release of $G\alpha_q$ from the beta-gamma subunit and activation of phospholipase C, which catalyzes the formation of DAG and IP3 from PIP2. Free

IP3 diffuses into the cytoplasm and activates IP3 receptors on the endoplasmic reticulum (ER) resulting in Ca^{2+} release from the ER into the cytoplasm. Elevated calcium levels lead to dephosphorylation of NFATc1 and rapid translocation from the cytoplasm to the nucleus [1,2].

Features

- Designed to assay compounds for their ability to modulate activation of the M3 receptor
- 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

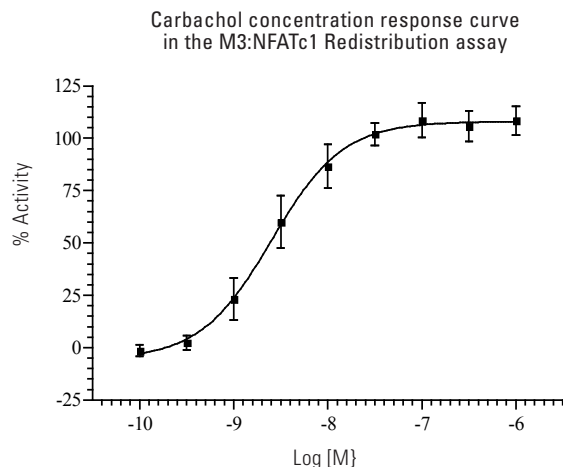


Figure 2. Carbachol concentration response in the M3:NFATc1 assay. The EC_{50} of carbachol is approximately 2.5 nM. Concentration response was measured in 9 point half log dilution series (n=16). Cells were treated with carbachol for 20 min. Cells were then fixed and nuclear translocation was measured using the Cellomics ArrayScan V^{TI} Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (300 nM carbachol) and negative control (0.25% DMSO).

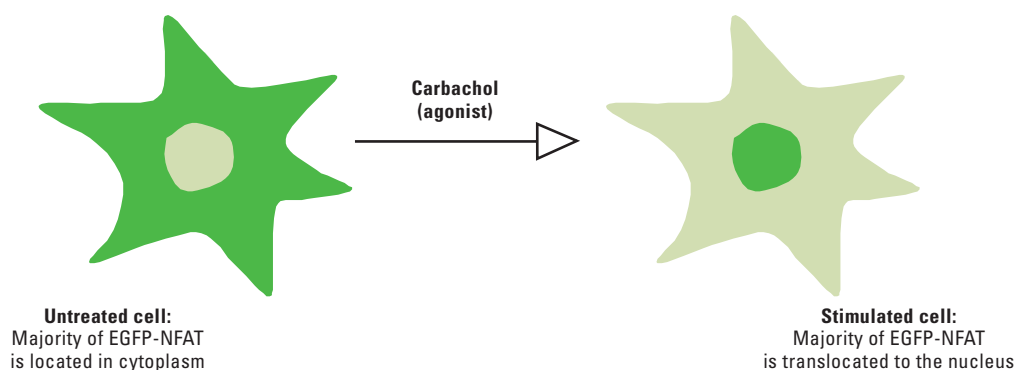


Figure 3. Illustration of the NFAT translocation in the M3:NFATc1 assay.

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Assay Details

Recombinant U2OS cells stably expressing human muscarinic acetylcholine receptor 3 (M3) and human NFATc1 fused to the C-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to screen for agonists causing M3 activation and thereby translocation of GFP-NFATc1 to the nucleus. Carbachol is used as reference compound. The M3:NFATc1 assay is validated with an average $Z' = 0.74 \pm 0.10$, suitable for both screening and profiling applications.

Imaging

The translocation of EGFP-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 M3:NFATc1 Redistribution assay is the translocation of EGFP-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 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 200 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.

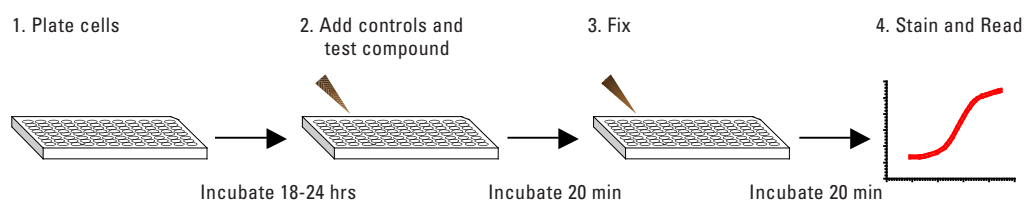


Figure 4. The M3:NFATc1 Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
073_01	M3: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	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO-K1	•	•	
046_01	β 2-AR:PKA Redistribution Assay	CHO-K1	•	•	
047_01	GlucagonR:PKA Redistribution Assay	CHO-K1	•	•	
067_01	S1P1:PKA Redistribution Assay	CHO-K1	•	•	
078_01	AT1R:NFATc1 Redistribution Assay	U2OS	•	•	
072_01	M2:PKA Redistribution Assay	CHO-K1	•	•	
048_01	NK1:NFATc1 Redistribution Assay	U2OS	•	•	
079_01	MCHR1:NFATc1 Redistribution Assay	U2OS	•	•	
088_01	M1:NFATc1 Redistribution Assay	U2OS	•	•	
081_01	MOR1:PKA Redistribution Assay	CHO-K1	•	•	
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			
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. Ishii et al. *Curr. Pharm. Des.*;12, 3573-81, 2006.
2. Rao, A. et al. *Annu. Rev. Immunol.*; 15, 707-747, 1997.

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