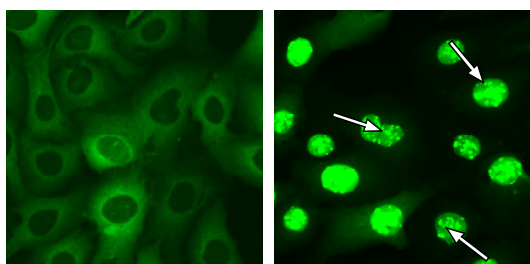


## Thermo Scientific AT<sub>1</sub>R:NFATc1 Redistribution<sup>®</sup> Assay

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 AT<sub>1</sub> receptor were treated with 300 nM angiotensin for 30 min (right panel) or untreated (DMSO control, left panel). Activation of the receptor leads to nuclear translocation of EGFP-NFATc1 (arrows), which can be detected by the image analysis algorithm.

### Thermo Scientific AT<sub>1</sub>R:NFATc1 Redistribution Assay

Angiotensin activates a wide spectrum of signaling responses via the Angiotensin II Type-1 receptor (AT<sub>1</sub>R) having immediate physiological effects on vasoconstriction and blood pressure regulation as well being implicated in inflammation, endothelial dysfunction, atherosclerosis, hypertension, and congestive heart failure. AT<sub>1</sub>R is a member of the superfamily of G protein-coupled receptors (GPCRs) and couples to G $\alpha$ q [1,2]. In this assay, the AT<sub>1</sub>R has been transfected into the GPCR Reporter Assay for Gq-coupled Receptors, where receptor activation leads to release of cytoplasmic Ca<sup>2+</sup>, which in turn induces NFATc1 translocation. Binding of an agonist to the extracellular parts of AT<sub>1</sub>R 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 IP<sub>3</sub> from PIP<sub>2</sub>. Free IP<sub>3</sub> diffuses into the cytoplasm, and activates IP<sub>3</sub> receptors on the endoplasmic reticulum

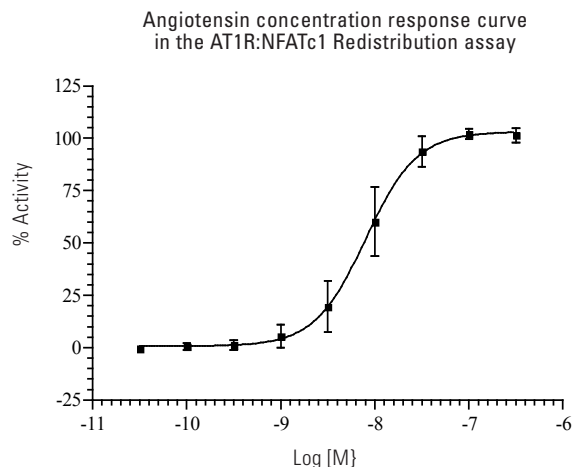
(ER) resulting in Ca<sup>2+</sup> release from the ER into the cytoplasm. Elevated calcium levels leads to dephosphorylation of NFATc1 and rapid translocation from the cytoplasm to the nucleus [3,4].

#### Features

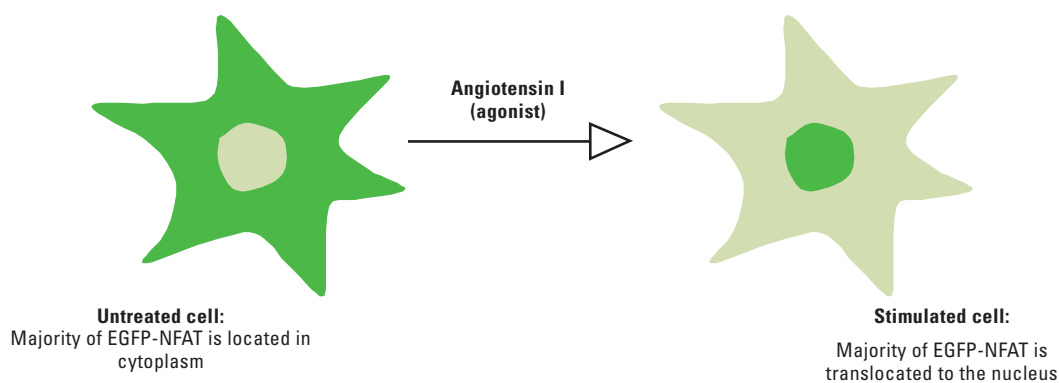
- Designed to assay compounds for their ability to modulate activation of the AT<sub>1</sub>R receptor
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications

#### 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. Angiotensin concentration response in the AT1R:NFATc1 assay.** The  $EC_{50}$  of angiotensin is  $\sim 10$  nM. Concentration response was measured in 9 point half log dilution series ( $n = 16$ ). Cells were treated with angiotensin for 30 min. Cells were then fixed and nuclear translocation was measured using the Cellomics ArrayScan V<sup>TI</sup> Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (300 nM angiotensin) and negative control (0.25% DMSO).



**Figure 3.** Illustration of the NFAT translocation in the AT1R:NFATc1 assay.

## Thermo Scientific AT1R:NFATc1 Redistribution<sup>®</sup> Assay

### Assay Details

Recombinant U2OS cells stably expressing human angiotensin II Type-1 receptor (AT1R) and human NFATc1 fused to the C-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to screen for agonists causing AT1R activation and thereby nuclear translocation of EGFP-NFATc1. Angiotensin I is used as reference compound. The AT1R:NFATc1 assay is validated with an average  $Z' = 0.83 \pm 0.05$ , suitable for both screening and profiling applications.

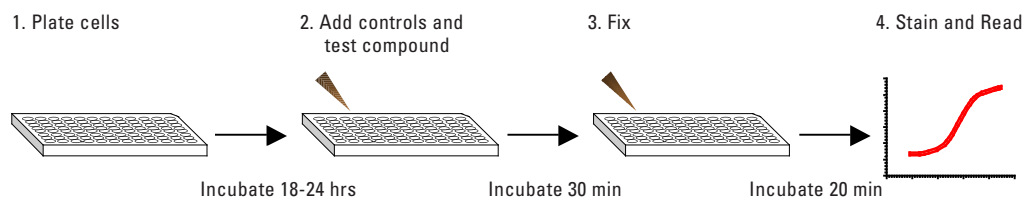
### Imaging

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 AT1R: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<sup>TI</sup>

This assay has been validated on the Cellomics Arrayscan V<sup>TI</sup> 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 250 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.



**Figure 4.** The AT1R:NFATc1 Redistribution assay is very easy and fast to perform.

### Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
078_01	AT1R: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	$\beta$ 2-AR:PKA Redistribution Assay	CHO-K1	•	•	
047_01	GlucagonR:PKA Redistribution Assay	CHO-K1	•	•	
067_01	S1P1:PKA Redistribution Assay	CHO-K1	•	•	
073_01	M3: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 <sup>TI</sup>	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

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