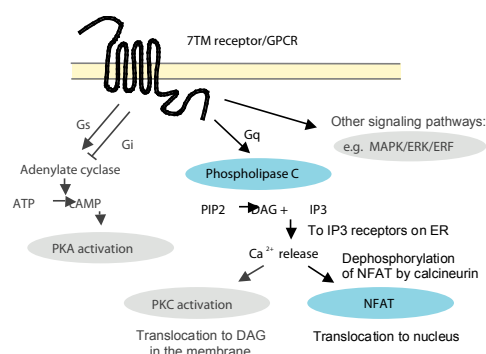


## Thermo Scientific NFATc1 Gq-coupled GPCR Reporter Redistribution<sup>®</sup> Assay

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: Schematic overview of GPCR signaling.** Gq-coupled GPCRs regulate the release of  $\text{Ca}^{2+}$  through activation of phospholipase C which leads to formation of DAG and  $\text{IP}_3$ . High levels  $\text{Ca}^{2+}$  is detected by calcineurin that dephosphorylates NFATc1 which induces cytoplasmic to nucleus translocation of NFATc1.

### Thermo Scientific NFATc1 Gq-coupled GPCR Reporter Redistribution Assay

The GPCR Reporter Assay for Gq-coupled Receptors uses NFATc1 translocation, caused by changes in the cytoplasmic  $\text{Ca}^{2+}$  concentration, as a reporter for activation of Gq-coupled GPCRs. Binding of an agonist to the extracellular parts of a Gq-coupled GPCR causes a conformational change in the intracellular face of the receptor, exchange of GDP for GTP on the alpha subunit ( $\text{G}\alpha_q$ ), and subsequently release of  $\text{G}\alpha_q$  from the beta-gamma subunit. GTP-bound  $\text{G}\alpha_q$  then activates phospholipase C, which catalyzes the formation of DAG and  $\text{IP}_3$ . Whereas DAG stays in the membrane, free  $\text{IP}_3$  diffuses into the cytoplasm, and activates  $\text{IP}_3$  receptors on the endoplasmic reticulum (ER) resulting in  $\text{Ca}^{2+}$  release from the ER into the cytoplasm. NFATc1 is a transcription factor involved in T-cell

signaling and tissue development, and its activity is controlled by the  $\text{Ca}^{2+}$ /Calmodulin-dependent phosphatase, calcineurin. Inactive NFATc1 resides in the cytosol, but in response to elevated calcium levels, NFATc1 is dephosphorylated by calcineurin, followed by rapid translocation of NFATc1 to the nucleus (Figure 1). In the nucleus, NFATc1 forms transcription complexes with other transcription factors such as AP-1, GATA4, GATA2 and MEF2 [1,2].

#### Features

- Designed to assay compounds for their ability to modulate activation of Gq-coupled GPCRs via NFATc1 translocation
- 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

1. Transient or stable transfection of Gq-coupled GPCR



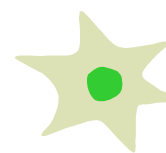
**Reporter cell line:**  
NFATc1 Gq GPCR reporter cell line. GFP-NFATc1 localizes to the cytoplasm

2. Optimize and run assay



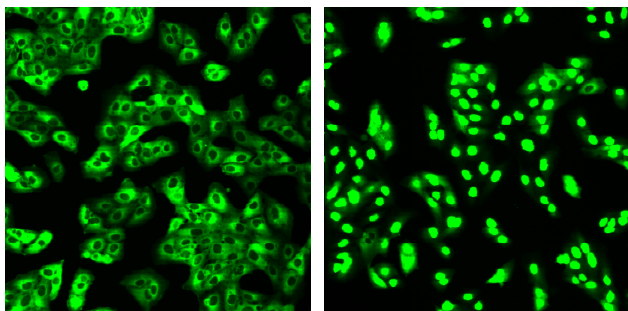
**GPCR reporter cells (untreated):**  
GPCR of choice expressed in the reporter cell line

GPCR agonist  
→

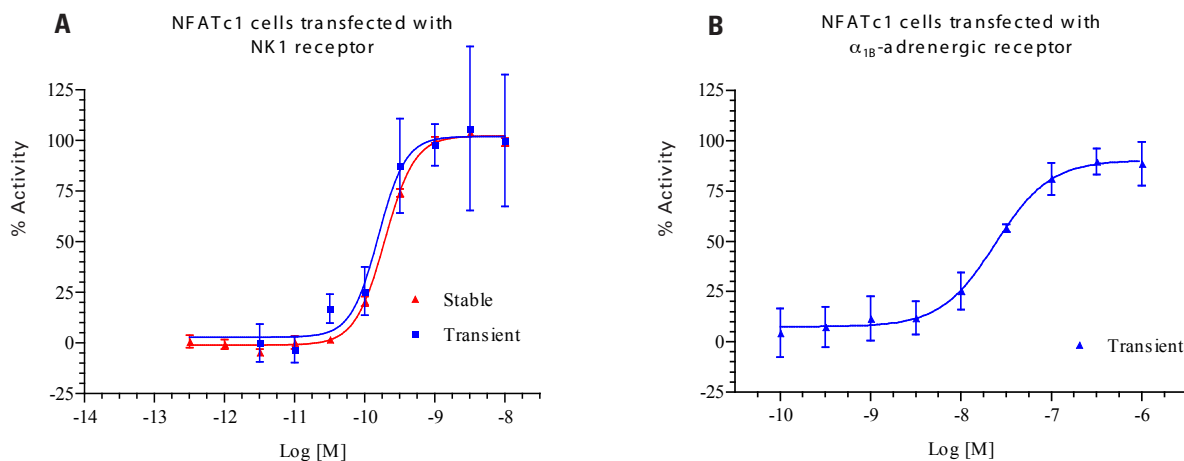


**GPCR reporter cells (stimulated):**  
GPCR activation causes nuclear translocation of GFP-NFATc1

**Figure 2:** Illustration of the use of the NFATc1 Redistribution reporter cell line resulting in the GFP-NFATc1 translocation event in response to activation of a user defined Gq-coupled GPCR.



**Figure 3. Images of GFP-NFATc1 expressing cells stably transfected with NK1 receptor.** Cells have been treated in the absence (DMSO control, left panel) or presence of receptor agonist, 10 nM substance P (right panel). Agonist treatment leads to cytoplasm to nucleus translocation of GFP-NFATc1.



**Figure 4. Concentration response curves for substance P and phenylephrine in the NFATc1 Gq GPCR reporter cell line transfected with NK1 receptor or  $\alpha_{1B}$ -adrenergic receptor.** A) NK1 receptor was transfected transiently or stably into the NFATc1 Gq GPCR reporter cell line. Transfected cells were treated with substance P in concentration response for 30 min. The  $EC_{50}$  is approximately 173 pM for transiently transfected cells and 193 pM for stably transfected cells. % activity was calculated relative to the positive (10 nM substance P) and negative control (0.25% DMSO) (n=4). B)  $\alpha_{1B}$ -adrenergic receptor was transfected transiently into the NFATc1 Gq GPCR reporter cell line. Transfected cells were treated with phenylephrine in concentration response for 30 min. The  $EC_{50}$  of phenylephrine is approximately 24 nM. % activity was calculated relative to the positive (300 nM phenylephrine) and negative control (0.25% DMSO) (n=4).

## Thermo Scientific NFATc1 Gq-coupled GPCR Reporter Redistribution<sup>®</sup> Assay

### Assay Details

Recombinant U2OS cells stably expressing human NFATc1 fused to the C-terminus of enhanced green fluorescent protein (EGFP). The NFATc1 Gq-coupled GPCR Redistribution cell line is developed to be used as a parental cell line to build specific GPCR assays after transfection of a GPCR of interest. Figure 2 illustrates the translocation of NFATc1 in response to agonist stimulation of a Gq-coupled GPCR. Test compounds causing translocation of NFATc1 to the nucleus are considered agonists for the GPCR, which has been transiently or stably transfected into the assay cell line.

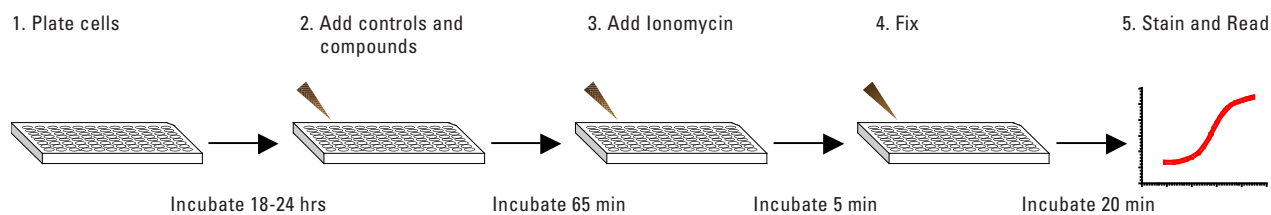
### Imaging

The translocation of NFAT 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 NFAT Redistribution assay is the translocation of NFAT 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>®</sup>

This assay has been developed on the Cellomics Arrayscan V<sup>®</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 200 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2 and ColocalizationV3.



**Figure 5:** The NFATc1 assay is very easy and fast to perform.

### Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
017_02	Gq-coupled GPCRs – 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
045_02	Gs/Gi-coupled GPCRs – 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			

### References

1. Rao, A. et al. *Annu. Rev. Immunol.*; 15, 707-747, 1997.
2. Masuda, E.S. et al. *Cell Signal.*; 10, 599-611, 1998.

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