

Thermo Scientific GRPR 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.

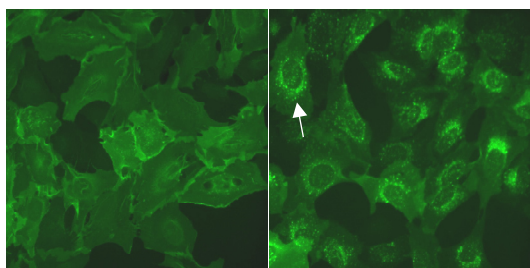


Figure 1. Internalization of GRPR-EGFP stimulated with GRP. Cells were treated with 100 nM GRP for 30 min (right panel) or untreated (DMSO control, left panel). Arrows indicate the GRPR-EGFP internalization that is detected by the image analysis algorithm.

Thermo Scientific GRPR Redistribution Assay

The gastrin releasing peptide (GRP) is the mammalian counterpart of the amphibian peptide bombesin (BB). GRP regulates critical gastrointestinal functions via the GRP receptor (GRPR). Activation of GRPR also stimulates cell proliferation and acts as a growth factor in the pathogenesis of many types of cancer. In addition, GRPR regulates several aspects of neuroendocrine function [1, 2]. GRPR is a Gq coupled G-protein-coupled receptor (GPCR), and when activated by GRP, GRPR mediates downstream activation of the phospholipase C signaling pathway. Following activation by GRP, GRPR and ligand are rapidly internalized into early endosomes. GRP remains in the intracellular space whereas GRPR recycles to the plasma membrane [3].

Features

- Designed to assay compounds for their ability to modulate internalization of GRPR
- 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

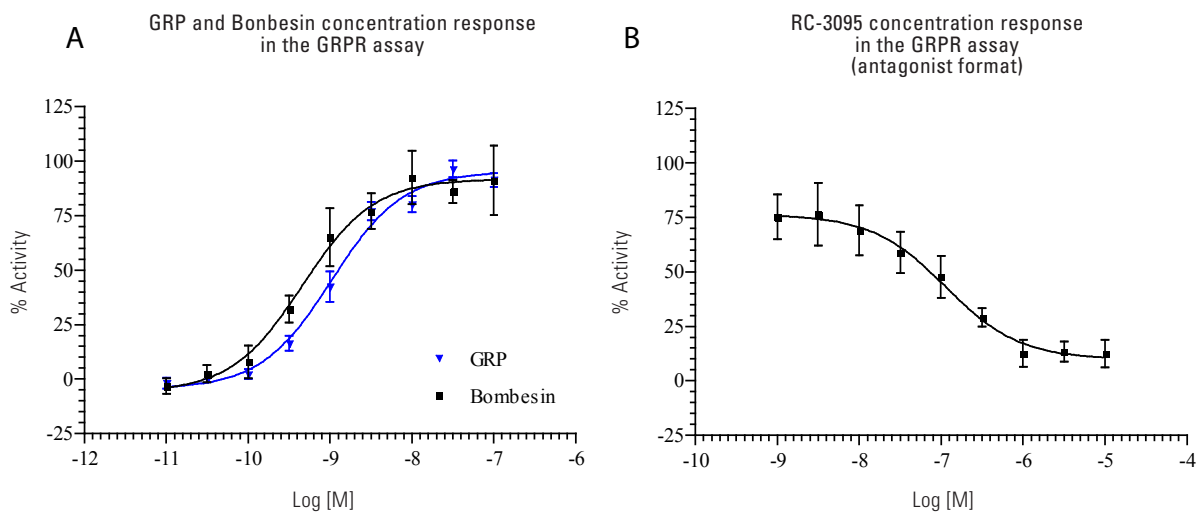


Figure 2. Concentration response curves in the GRPR assay: A) GRP and Bombesin concentration response in the GRPR agonist assay ($n = 8$). The EC_{50} values of GRP and bombesin are ~ 1 nM and ~ 0.5 nM respectively. Concentration response was measured in 9 point half log dilution series. Cells were treated with compound for 30 min. Cells were then fixed and receptor internalization was measured using the Cellomics ArrayScan V^{II} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (100 nM GRP) and negative control (0.25% DMSO). **B)** RC-3095 concentration response in the GRPR assay run in antagonist format ($n = 8$). Cells were treated with 10 nM GRP in the presence of a half log dilution series of RC-3095 for 30 min. Cells were then fixed and analyzed on the Cellomics ArrayScan V^{II} Reader. The EC_{50} of RC-3095 is ~ 100 nM.

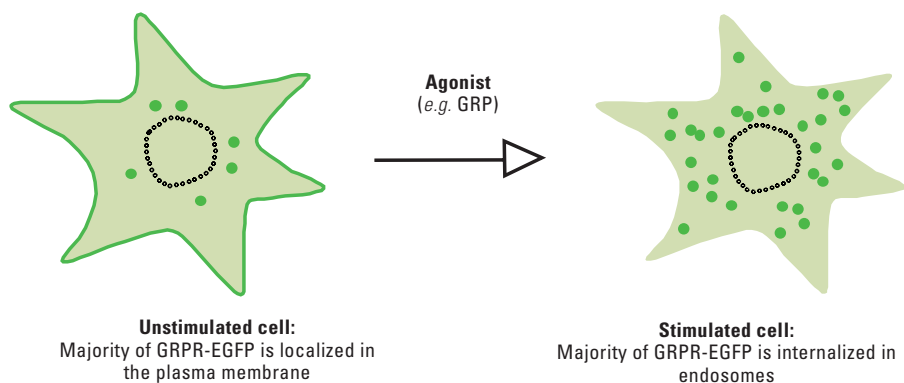


Figure 3. Illustration of the GRPR translocation event.

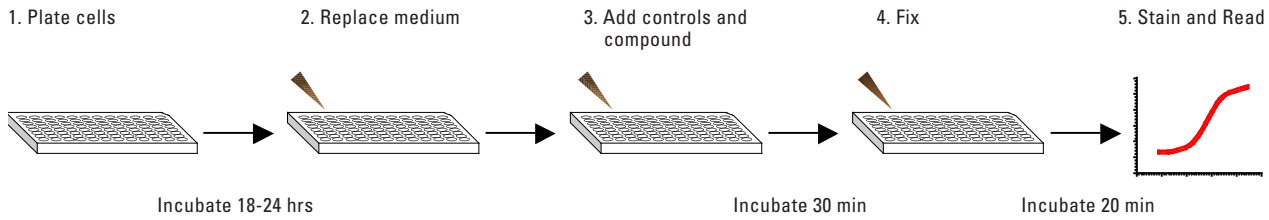


Figure 4. The GRPR Redistribution assay is very easy and fast to perform.

Thermo Scientific GRPR Redistribution® Assay

Assay Details

Recombinant U2OS cells stably expressing human gastrin releasing peptide receptor (GRPR) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to screen for agonists of GRPR activation by monitoring the internalization of a membrane-localized GRPR-EGFP fusion protein. The assay may be re-formatted to antagonist format by using GRP as agonist and RC-3095 as a BB/GRP receptor reference antagonist. The GRPR assay is validated with an average $Z' = 0.55 \pm 0.10$, suitable for both screening and profiling applications.

Imaging

The translocation of GRPR-EGFP 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 20x objective or higher magnification. The primary output in the GRPR Redistribution assay is the formation of spots in the cytoplasm. The data analysis should therefore report an output that corresponds to number, area or intensity of spots in the cytoplasm.

Imaging on Thermo Scientific Cellomics ArrayScan V^{TI}

This assay has been validated on the Cellomics Arrayscan V^{TI} using a 20x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the SpotDetectorV3 BioApplication. The output parameter used was SpotCountPerObject. 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 CompartmentalAnalysisV2 and ColocalizationV3.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
094_01	GRPR 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
067_01	CXCR4 Redistribution Assay	U2OS	•	•	
093_01	CRTH2 Redistribution Assay	U2OS	•	•	
054_01	MCH1 Redistribution Assay	U2OS	•		
039_01	S1P ₁ Redistribution Assay	U2OS	•	•	•
095_01	S1P ₃ Redistribution Assay	U2OS	•		•
086_01	M1 Redistribution Assay	U2OS	•		
075_01	M2 Redistribution Assay	U2OS	•	•	
076_01	M3 Redistribution Assay	U2OS	•	•	
057_01	MC4 Redistribution Assay	U2OS	•		
053_01	FSHR Redistribution Assay	U2OS	•	•	
051_01	CB1 Redistribution Assay	U2OS	•	•	•
061_01	CB2 Redistribution Assay	U2OS	•		•
097_02	GLP1R Redistribution Assay	U2OS	•	•	
017_02	Gq-coupled GPCRs – NFATc1 Redistribution Assay	U2OS	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO-K1	•	•	
088_01	M1:NFATc1 Redistribution Assay	U2OS	•	•	
072_01	M2:PKA Redistribution Assay	CHO-K1	•	•	
073_01	M3:NFATc1 Redistribution Assay	U2OS	•	•	
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. Cornelio DB et al., *Ann Oncol.* 9:1457-66, 2007.
2. Roesler R et al., *CNS Neurol Disord Drug Targets* 5 (2):197-204, 2006.
3. Grady EF et al., *JBC.* 270, 4603-4611, 1995.

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