Product Specifications

Thermo Scientific GLP1R 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.

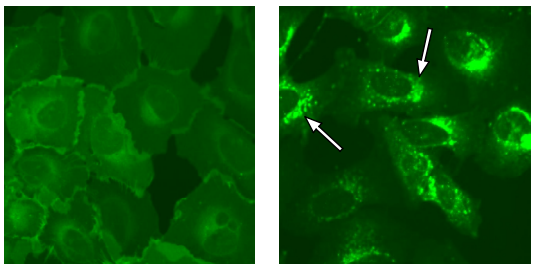

Figure 1. Internalization of GLP1R-EGFP stimulated with GLP1. DMSO control (left panel) and cells treated with 100 nM GLP1 for 1 hr (right panel). Arrows indicate the GLP1R-EGFP internalization that is detected by the image analysis algorithm.

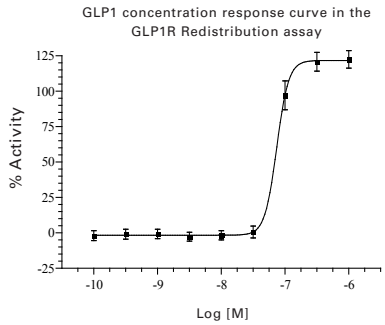

Figure 2. GLP1 concentration response in the GLP1R assay. Concentration-response curves of wortmmanin in the Akt1 . Concentration response was measured in 9 point half log dilution series (n=16). Cells were incubated with GLP1 for 1 hr. Cells were then fixed and internalization was measured using the Cellomics ArrayScan V^{TI} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (100 nM GLP1) and negative control (0.25% DMSO). The EC_{50} of GLP1 is ~70 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

Thermo Scientific GLP1R Redistribution Assay

Glucagon-like peptide 1 (GLP1) is an intestinal hormone. It is secreted into the blood by the intestinal L cells in response to food intake. GLP1 receptor (GLP1R) is a G-protein coupled receptor of the glucagons/secretin/vasoactive intestinal peptide receptor subfamily. GLP1R couples to Gs and receptor activation leads to an increase in cellular cAMP. Upon binding of ligand the receptor is internalized/endocytosed and recycled to the cell surface. GLP1R is expressed on the surface of pancreatic β-cells and ligand binding results in activation of adenylate cyclase and insulin release. Additional effects of GLP1R activation include suppression of glucagon secretion and reduction in appetite, food intake and gastric emptying. These functions make the GLP1 receptor a promising target for treatment of type 2 diabetes using GLP1R agonists [1-3].

Features

- Designed to assay compounds for their ability to internalize GLP1 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

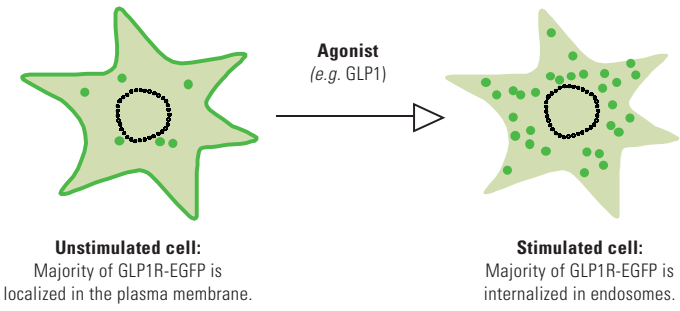

Figure 3. Illustration of the GLP1R translocation event.

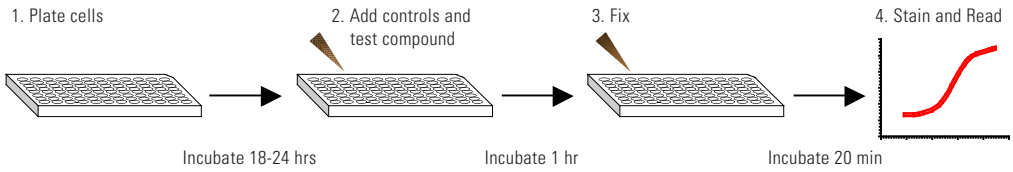

Figure 4. The GLP1R Redistribution assay is very easy and fast to perform.

Thermo Scientific GLP1R Redistribution® Assay

Assay Details

Recombinant U2OS cells stably expressing human GLP1 receptor fused to the N-terminus of enhanced green fluorescent protein (EGFP). The GLP1R assay is designed to screen for agonists causing internalization of GLP1R. GLP1 is used as reference compound in the assay, and ligands/compounds are assayed for their ability to induce GLP1R internalization by a spot detecting image analysis algorithm. The GLP1R assay is validated with an average Z′ = 0.76 ± 0.04, suitable for screening and profiling applications.

Imaging

The translocation of GLP1R-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 GLP1R 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 SpotTotalAreaPerObject. The minimally acceptable number of cells used for image analysis in each well was set to 100 cells.

Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
097_01	GLP1R 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
023_01	GLUT4 Redistribution Assay	CHO	•		
089_01	GLUT1 Redistribution Assay	CHO	•		
065_01	TORC2 Redistribution Assay	U2OS	•	•	
047_01	GlucagonR:PKA Redistribution Assay	CHO	•	•	
045_02	Gs/Gi-coupled GPCRs – PKA Redistribution Assay	CHO	•	•	
8407101	Cellomics Phospho-GSK-3 Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
8404701	Cellomics PKA and Phospho-CREB Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8405201	Cellomics Phospho-S6 Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
8405301	Cellomics Phospho-4E-BP1 Detection 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. Widmann C et al., *Molecular endocrinology,* 11, 1094-1102, 1997.
2. Jorgensen R et al., *Molecular endocrinology*, 19, 812-823, 2005.
3. Drucker DJ, *Diabetes Care*, 26, 2929-2940, 2003.

Life Science Reasearch

BioImage Products
2650 Crescent Drive
Suite 100
Lafayette, CO 80026

303-604-9499
303-604-3370 fax

bioimage@thermofisher.com
www.thermo.com/hcs

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