

Thermo Scientific GLUT1 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.

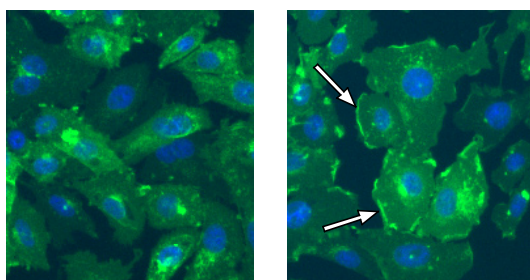


Figure 1. Translocation of GLUT1-EGFP in response to insulin. Cells were treated with 100 nM insulin for 5 min (right panel) or untreated (DMSO control, left panel). Arrows indicate the GLUT1-EGFP translocation from the cytoplasm to the plasma membrane that is detected by the image analysis algorithm.

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Glucose transporters (GLUT) are a family of membrane proteins which includes twelve members, numbered 1-12. A thirteenth member of the GLUT family is the myoinositol transporter, HMIT1. GLUT1 is highly expressed in erythrocytes and brain, while GLUT4 is responsible for insulin-regulated glucose transport in adipose tissues, heart muscles, and skeletal muscles [1]. Insulin triggers translocation of GLUT1 from intracellular membrane compartments to the plasma membrane. This translocation involves the PI3K pathway, and GLUT1 translocation is inhibited by the specific PI3K inhibitor wortmannin [2].

Features

- Designed to assay compounds for their ability to modulate translocation of GLUT1 to the plasma membrane
- 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

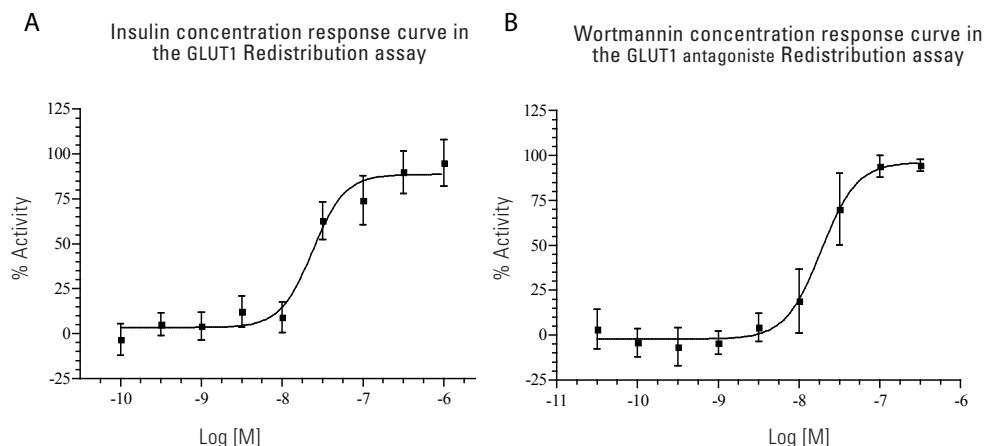


Figure 2. Concentration response curves in the GLUT1 assay: A) Insulin concentration response in the GLUT1 agonist assay ($n = 16$). The EC_{50} is ~ 24 nM. Concentration response was measured in 9 point half log dilution series. Cells were treated with insulin for 5 min. Cells were then fixed and GLUT1 translocation was measured using the Cellomics ArrayScan V^{Hi} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (1 μ M insulin) and negative control (0.125% DMSO). **B)** Wortmannin concentration response in the GLUT1 assay run in antagonist format ($n = 16$). Cells were preincubated for 20 min with a half log dilution series of test compound (wortmannin), followed by a 5 minute treatment with 100 nM insulin. Cells were then fixed and analyzed on the Cellomics ArrayScan V^{Hi} Reader. % activity was calculated relative to the positive (150 nM wortmannin) and negative control (0.25% DMSO). The EC_{50} of wortmannin is ~ 17 nM.

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Assay Details

Recombinant CHO_{HR} cells stably expressing human GLUT1 fused to the N-terminus of enhanced green fluorescent protein (EGFP). The GLUT1 Redistribution assay can be used to identify compounds that modulate translocation of GLUT1 and thereby affect glucose uptake or as a selection/de-selection assay for the GLUT4 Redistribution assay. The assay is available in both agonist and antagonist format. Insulin is used as a reference agonist. In the antagonist configuration the assay can be used to screen for compounds that mimic the effect of wortmannin on insulin-induced GLUT1 translocation. The GLUT1 assay is validated with an average $Z' = 0.34 \pm 0.06$, suitable for profiling applications.

Imaging

The translocation of GLUT1-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 10x objective or higher magnification. The

primary output in the GLUT1 Redistribution assay is the translocation of GLUT1-EGFP from an intracellular location to the plasma membrane. The data analysis should therefore report an output that corresponds to number, area, or intensity of these spots in the membrane.

Imaging on Thermo Scientific Cellomics ArrayScan V^{Hi}

This assay has been validated on the Cellomics Arrayscan V^{Hi} using a 10x objective (0.63X coupler), High Resolution images, 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 700 cells. Other BioApplications that can be used for this assay include ColocalizationV3 and CytoCellMemTransV2.

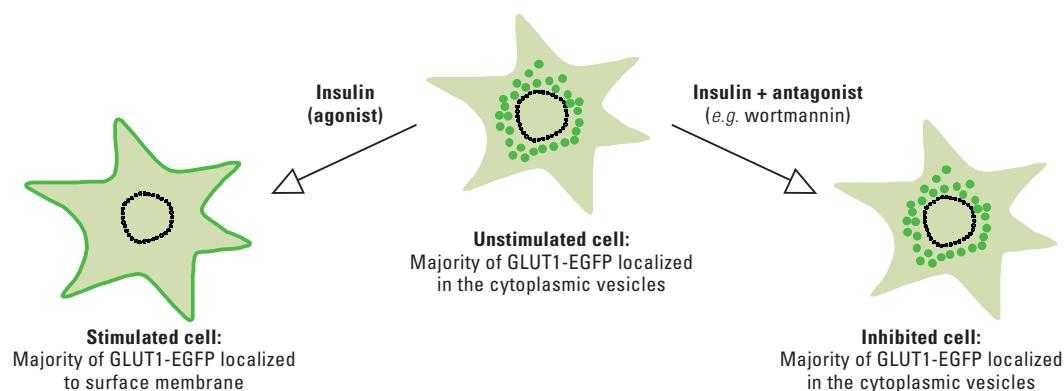
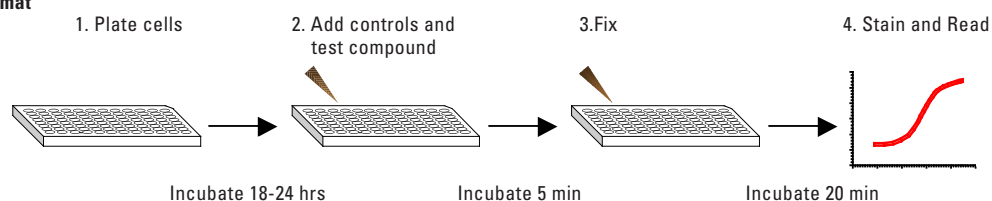


Figure 3. Illustration of the GLUT1 translocation event.

A. Agonist format



B. Antagonist format

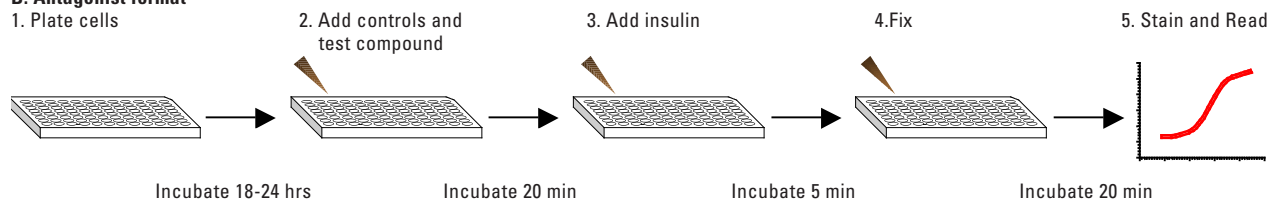


Figure 4. The GLUT1 Redistribution assay is very easy and fast to perform in both agonist and antagonist format.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
089_01	GLUT1 Redistribution Assay	CHO	•		

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	•	•	
8407101	Cellomics Phospho-GSK-3 Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
8404101	Cellomics Phospho-AKT Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8403601	Cellomics Beta-Catenin Activation 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 ^{II}	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

References

1. Pascual JM. *Eur J Endocrinol.* , 150, 627-633, 2004.
2. Perrini S. *Diabetes*, 53, 41-52, 2004

