

Thermo Scientific GLUT4 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.

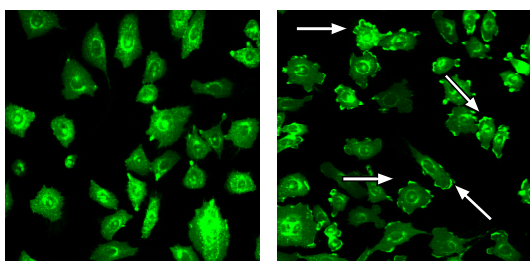


Figure 1. Translocation of GLUT4-EGFP in response to insulin. Cells were treated with 50 nM insulin for 5 min (right panel) or vehicle control (left panel). Arrows indicate the GLUT4-EGFP translocation from cytoplasmic vesicles to the plasma membrane that is detected by the image analysis algorithm.

Thermo Scientific GLUT4 Redistribution Assay

The GLUT4 glucose transporter is essential for insulin-stimulated glucose uptake, and is expressed primarily in adipose and muscle tissues. In response to insulin, intracellular GLUT4 vesicles translocate to cytoskeletal structures near the cell surface, followed by fusion of the GLUT4 vesicles with the plasma membrane. Defective uptake of glucose via GLUT4 in response to insulin is a central feature of obesity and type 2 diabetes [1,2].

Features

- Designed to assay compounds for their ability to modulate GLUT4 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

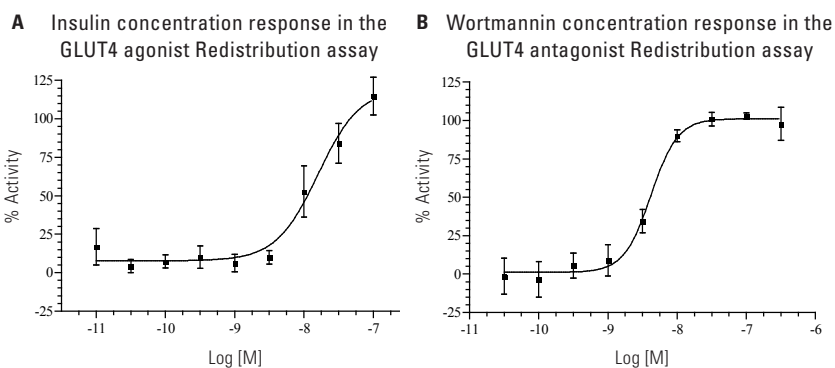


Figure 2. Concentration response curves in the GLUT4 assay: A) Insulin concentration response in the GLUT4 agonist assay (n = 8). The EC₅₀ is ~16 nM. Cells were treated with insulin for 5 min. Cells were then fixed and GLUT4 translocation was measured using the Celloomics ArrayScan V^{TI} Reader and the CytoCellMemTransV2 BioApplication. % activity was calculated relative to the positive (50 nM insulin) and negative control. **B)** Wortmannin concentration response in the GLUT4 assay run in antagonist format (n = 8). Cells were preincubated for 20 min with a half log dilution series of wortmannin, followed by a 5 minute treatment with 20 nM insulin. Cells were then fixed and analyzed on the Celloomics ArrayScan V^{TI} Reader. % activity was calculated relative to the positive (100 nM wortmannin) and negative control. The EC₅₀ of wortmannin is ~4 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

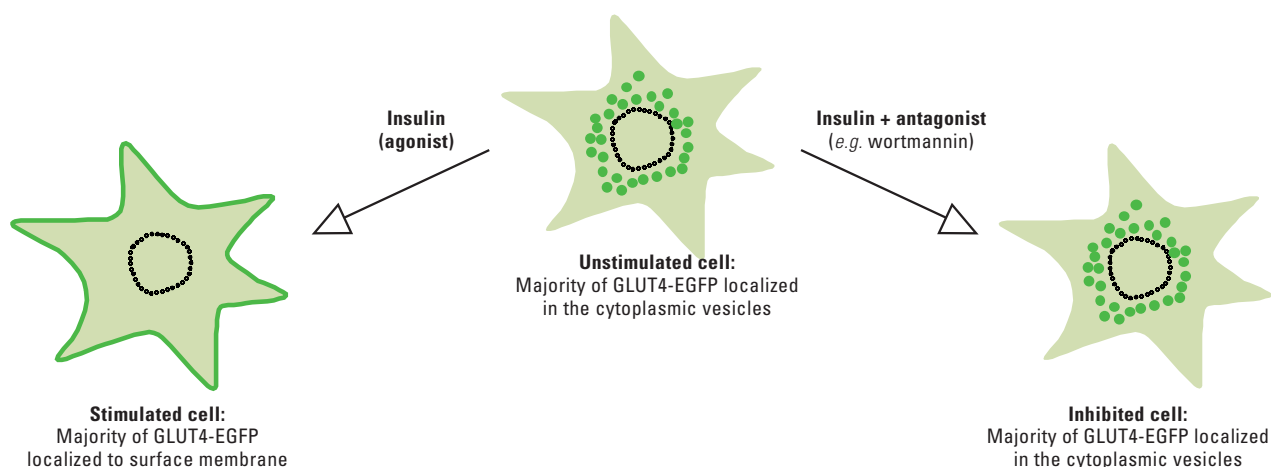


Figure 3. Illustration of the GLUT4 translocation event.

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Assay Details

Recombinant CHO_hIR cells stably expressing human GLUT4 fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is available in both agonist and antagonist format. The agonist assay can be used to identify compounds that modulate translocation of GLUT4 and thereby affect glucose uptake. Insulin is used as reference agonist. Furthermore, the assay can potentially be formatted to screen for compounds that enhance the translocation of GLUT4 in response to insulin (*i.e.* insulin sensitizers). The antagonist assay can be used to identify compounds that antagonize insulin-stimulated GLUT4 translocation and thereby affect glucose uptake. In the antagonist format, the PI3K inhibitor wortmannin is used as reference antagonist. The GLUT4 assay is validated with an average $Z' = 0.49 \pm 0.12$ (agonist mode) and $Z' = 0.68 \pm 0.09$ (antagonist mode), suitable for both screening and profiling applications.

Imaging

The translocation of GLUT4-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 GLUT4 Redistribution assay is the translocation of GLUT4-EGFP from cytoplasmic vesicles to membrane spots. 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[®]

This assay has been validated on the Cellomics ArrayScan V[®] using a 10x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the CytoCellMemTrans.V2 BioApplication. The output parameter used was MEAN_MemRingAvgIntenRatio. The minimally acceptable number of cells used for image analysis in each well was set to 800 cells. Other BioApplications that can be used for this assay include ColocalizationV3.

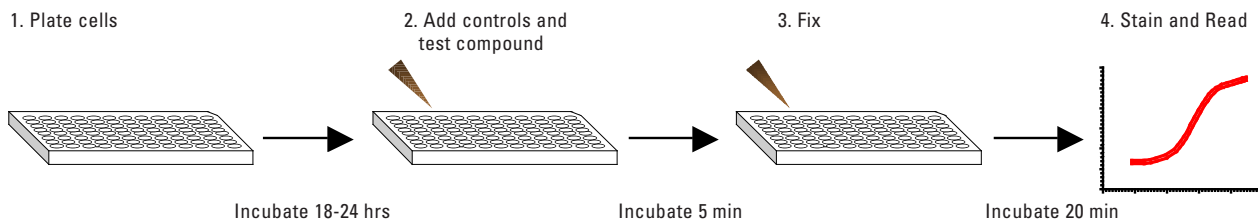
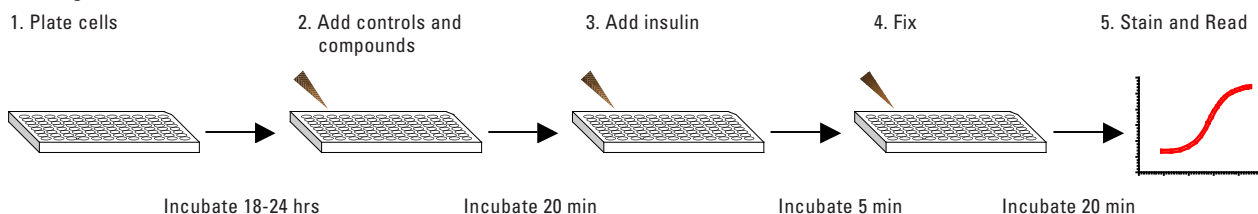
A. Agonist format**B. Antagonist format**

Figure 4. The GLUT4 Redistribution assay is very easy and fast to perform in both agonist and antagonist format.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
023_01	GLUT4 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
089_01	GLUT1 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 (orange) 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 ^{TI}	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

References

1. James DE. *J Clin Invest.* 115,219-21, 2005
2. Pessin JE et al. *J Biol Chem.* 274, 2593-2596, 1999.

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