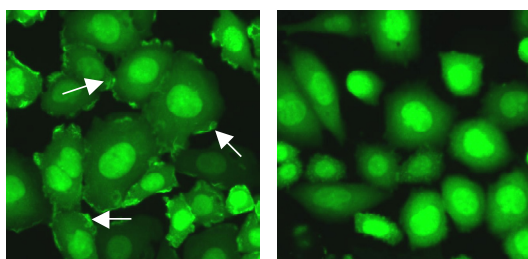


## Thermo Scientific Akt1 Redistribution<sup>®</sup> Assay

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.** Images illustrating IGF-I-treated cells in the absence (DMSO control, left panel) or presence of 300 nM wortmannin for 4 min (right panel). Arrows indicate IGF-I-mediated membrane translocation detected by the image analysis algorithm.

### Thermo Scientific Akt1 Redistribution Assay

The serine/threonine kinase Akt, also known as protein kinase B (PKB), is essential for cell signaling downstream of growth factors, cytokines, and other cellular stimuli. Akt thereby contributes to a variety of cellular functions such as cell survival, growth, proliferation, angiogenesis, metabolism, and migration. The Akt/PKB family consists of three highly homologous members known as Akt1/PKB $\alpha$ , Akt2/PKB $\beta$ , and Akt3/PKB $\gamma$  in mammalian cells. The Akt signaling pathway is activated by various growth factors (*e.g.* insulin, IGF-I, EGF) and upon activation Akt translocates from the cytoplasmic space to the inner surface of the plasma membrane, where it binds to specific phospholipids produced by phosphatidylinositol 3-kinase (PI3K) at the membrane [1]. Deregulation of Akt such as aberrant loss or gain of Akt activation often results in a variety of complex diseases, including type-2 diabetes and cancer, making Akt a viable drug target for cancer therapy [2].

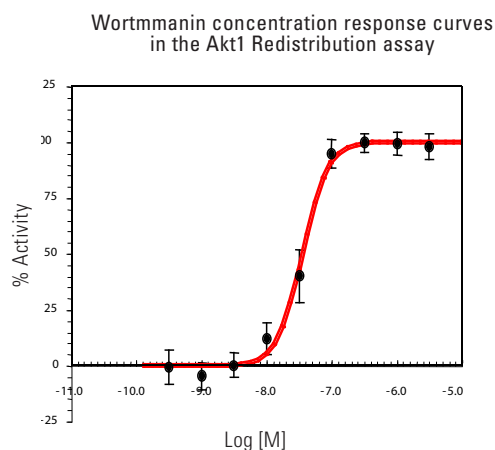
The Akt1 Redistribution assay monitors translocation of GFP-Akt1 fusion protein from the cytoplasm to the plasma membrane. Insulin-like growth factor-I (IGF-I) is used as reference agonist, and compounds are assayed for their ability to inhibit IGF-I-stimulated membrane translocation of Akt1. The PI3K inhibitor wortmannin [2,3] is used as reference antagonist, having an EC<sub>50</sub> value of ~35 nM in the assay. Compounds interfering with membrane translocation of Akt1 can be analyzed for isoform selectivity using the reagents in the table of Related Products below.

### Features

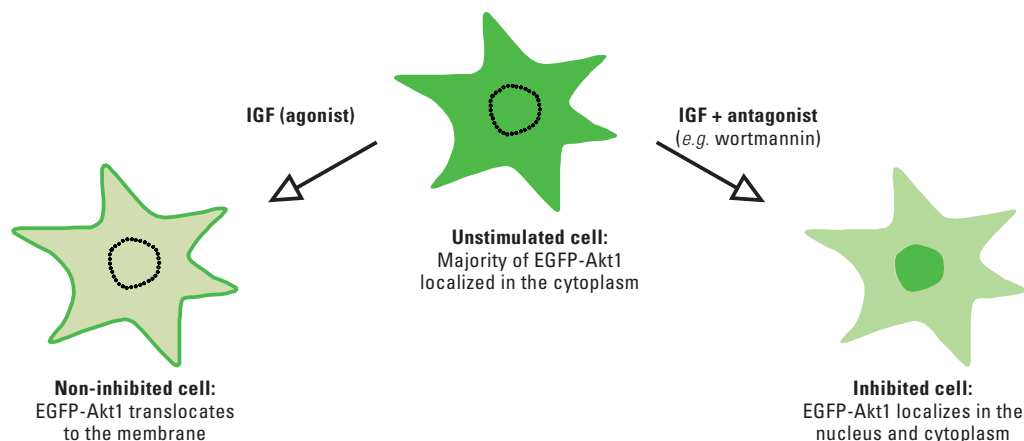
- Designed to assay compounds for their ability to modulate membrane translocation of the Akt1-domain
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications

### 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. Effect of wortmannin in the Akt1 Redistribution assay.** Concentration-response curves of wortmannin in the Akt1 Redistribution assay with 4 min compound incubation in medium containing 0.05 % FBS. The  $EC_{50}$ -value of wortmannin is approximately 35 nM,  $n = 16$ . Cells were pre-incubated with 100 nM IGF-1 for 60 min. and treated with wortmannin for 4 min. Cells were then fixed and membrane translocation was measured using the Cellomics ArrayScan V<sup>TI</sup> Reader and the CytoCellMemTrans.V2 BioApplication. % activity was calculated relative to the positive (500 nM wortmannin) and negative control (0.25% DMSO).



**Figure 3.** Illustration of the Akt1 translocation event.

## Thermo Scientific Akt1 Redistribution<sup>®</sup> Assay

### Assay Details

Recombinant CHO-hIR cells stably expressing human Akt1 fused to the C-terminus of enhanced green fluorescent protein (EGFP). Insulin-like growth factor-I (IGF-I) is used as a reference agonist, and compounds are assayed for their ability to inhibit IGF-I-stimulated membrane translocation of Akt1. The PI3K inhibitor wortmannin [1, 2] is used as reference antagonist. Compounds inhibiting IGF-I-induced cytoplasm to membrane translocation of Akt1 could: interfere directly with Akt1 translocation (*e.g.* PH-domain binding), act upstream of Akt1, or inhibit Akt1 kinase activity and thereby modulate Akt1 translocation. In general, Akt1 kinase inhibitors that do not modulate translocation of Akt1 (*e.g.* competitive catalytic site inhibitors) are predicted to be negative in the Akt1 assay. The Akt1 assay is validated with an average  $Z' = 0.57 \pm 0.11$ , suitable for both screening and profiling applications.

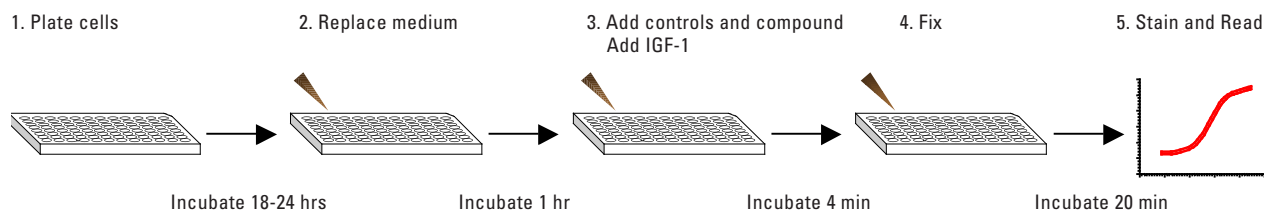
### Imaging

The translocation of EGFP-Akt1 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 Akt1 Redistribution assay is the translocation of EGFP-Akt1 from cytoplasm 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<sup>TI</sup>

This assay has been validated on the Cellomics ArrayScan V<sup>TI</sup> using a 20x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC and the CytoCellMemTrans.V2 BioApplication. The output parameter used was MEAN\_%MemColoc. 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 ColocalizationV3.



**Figure 4.** The Akt1 Redistribution assay is very easy and fast to perform.

### Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
085_01	Akt1 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
006_01	Akt1-PH Redistribution Assay	CHO	•	•	•
011_02	Akt2 Redistribution Assay	CHO	•	•	•
012_01	Akt3 Redistribution Assay	CHO	•	•	
008_01	FKHR (FOXO1) Redistribution Assay	U2OS	•	•	•
009_02	FKHRL1 (FOXO3) Redistribution Assay	U2OS	•	•	•
090_01	AFX (FOXO4) Redistribution Assay	U2OS	•	•	
013_01	PDK1 Redistribution Assay	CHO	•		
8404102	Cellomics Phospho-AKT Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8407101	Cellomics Phospho-GSK-3 Detection 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 <sup>TI</sup>	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

### References

1. Manning BD & Cantley LC, *Cell* 129, 1261-1274, 2007.
2. Cheng JQ et al., *Oncogene* 24, 7482-7492, 2005.
3. Acaro A & Wymann MP, *Biochem J* 296, 297-301, 1993.
4. Burgering BM & Coffey PJ, *Nature* 376, 599-602, 1995.

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