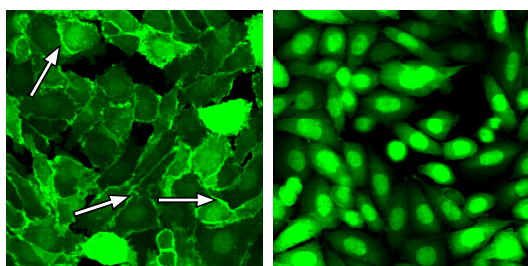


## Thermo Scientific Akt1-PH Redistribution<sup>®</sup> Assay

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. Membrane translocation of EGFP-Akt1-PH.** Cells were treated with 100 nM IGF-1 without (DMSO control, left panel) and with addition of 500 nM wortmannin (right panel). Arrows indicate IGF-1 induced membrane translocation of EGFP-Akt1-PH detected by the image analysis algorithm.

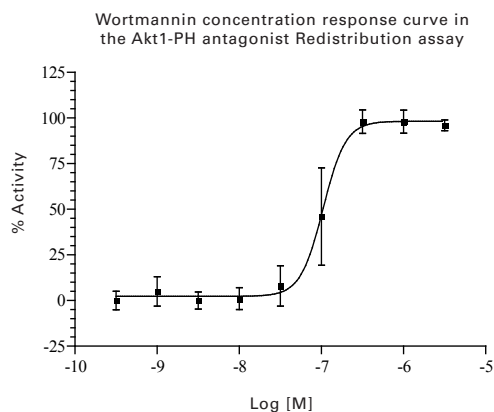
### Thermo Scientific Akt1-PH Domain Redistribution Assay

The Akt1/PKB $\alpha$  signaling pathway is activated by various growth factors (e.g. insulin, IGF-I) and mediates cell survival signals. Upon activation of the pathway, Akt1 translocates from the cytoplasm to the plasma membrane, due to the production of specific phospholipids by phosphatidylinositol 3-kinase (PI3K) in the membrane. The Akt1-PH domain Redistribution assay monitors translocation of the EGFP-Akt1-PH fusion protein from the cytoplasm to the plasma membrane. The assay is designed as a companion assay to be used in combination with our full length

Akt1 Redistribution assay (see related products) to elucidate the significance of the PH-domain of Akt1 for small molecule inhibitors of Akt1 membrane translocation.

#### Features

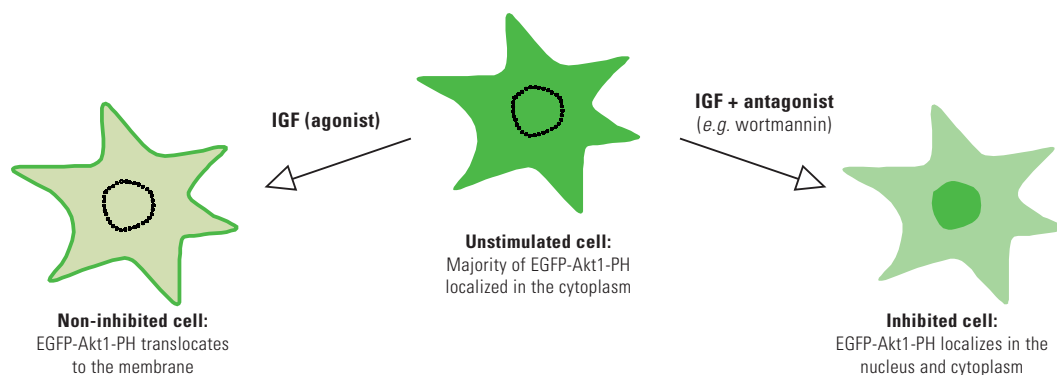
- Designed to assay compounds for their ability to modulate membrane translocation of the Akt1-PH domain
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications



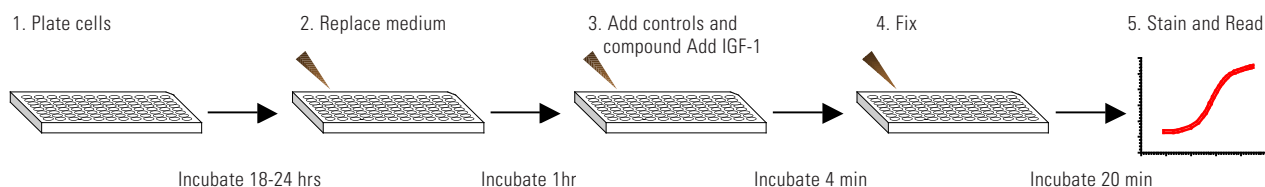
**Figure 2. Concentration response curve in the Akt1-PH antagonist assay.** Wortmannin concentration response curve in the Akt1-PH antagonist Redistribution assay stimulated by 100 nM IGF-1 (n=16). The EC<sub>50</sub> of wortmannin is 105 nM. Concentration response was measured in 9 point half log dilution series. 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).

#### 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 Akt1-PH domain translocation event.



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

## Thermo Scientific Akt1-PH Redistribution® Assay

### Assay Details

Recombinant CHOhr cells stably expressing human Akt1-PH domain 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 the Akt1-PH domain. The PI3K inhibitor wortmannin [1, 2] is used as reference antagonist. Compounds inhibiting IGF-I-induced cytoplasm to membrane translocation of Akt1 could be interfering directly with PH-domain binding of Akt1 or act upstream of Akt1. The Akt1-PH assay is validated with an average  $Z' = 0.68 \pm 0.09$ , suitable for both screening and profiling applications.

### Imaging

The translocation of EGFP-Akt1-PH 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-PH Redistribution assay is the translocation of EGFP-Akt1-PH 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.

## Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
006_01	Akt1-PH 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 Thermo Scientific Managed Services.

## Related Thermo Scientific Products

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
085_01	Akt1 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	•		
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			
8404601	Cellomics Cell Cycle I 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>1</sup>	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

## References

1. Acaro A & Wymann MP, *Biochem J* 296, 297-301, 1993.
2. Burgering BM & Coffey PJ, *Nature* 376, 599-602, 1995.

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