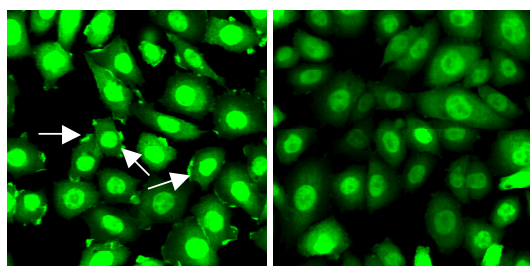


## Thermo Scientific Akt3 Redistribution<sup>®</sup> Assay

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 Akt3-EGFP.** Cells were treated with 100 nM IGF-1 without (DMSO control, left panel) and with (right panel) addition of 300 nM wortmannin. Arrows indicate IGF-1 induced membrane translocation of Akt3-EGFP detected by the image analysis algorithm.

### Thermo Scientific Akt3 Redistribution Assay

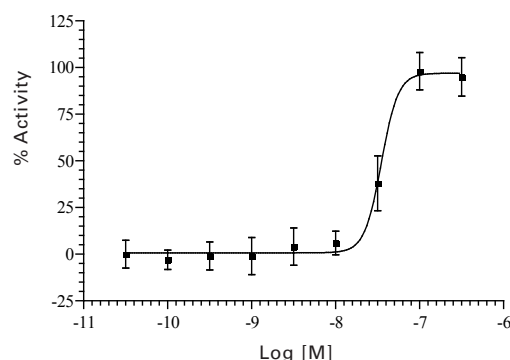
The Akt/protein kinase B (PKB) family of serine/threonine-specific protein kinases comprises three highly homologous members in mammalian cells (Akt1/PKB $\alpha$ , Akt2/PKB $\beta$  and Akt3/PKB $\gamma$ ). The Akt family members are activated by diverse stimuli such as hormones and growth factors (*e.g.* insulin and IGF-I). The Akt protein kinases function within the phosphoinositide 3-kinase (PI3K) signaling pathway. PI3K generates phosphatidylinositol-3,4,5-trisphosphate (PIP3), a lipid second messenger essential for the translocation of Akt/PKB to the plasma membrane. Following translocation to the membrane, Akt is phosphorylated and activated by

the phosphoinositide-dependent kinase-1 (PDK-1). Activated Akt phosphorylates and regulates the function of many cellular proteins essential for metabolism, apoptosis, and proliferation.

#### Features

- Designed to assay compounds for their ability to modulate membrane translocation of Akt3
- 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

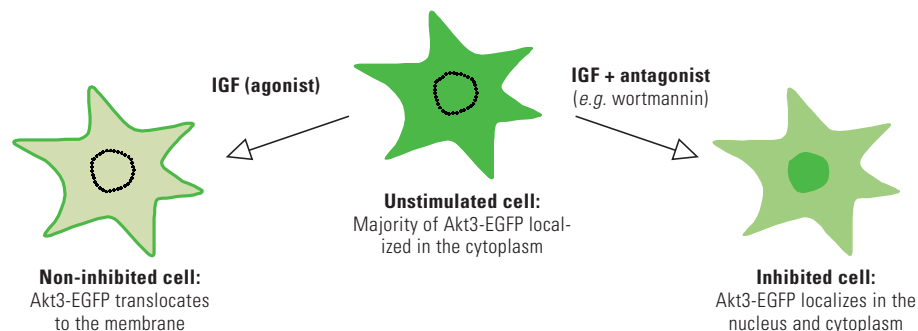
Wortmannin concentration response curve in the Akt3 antagonist Redistribution assay



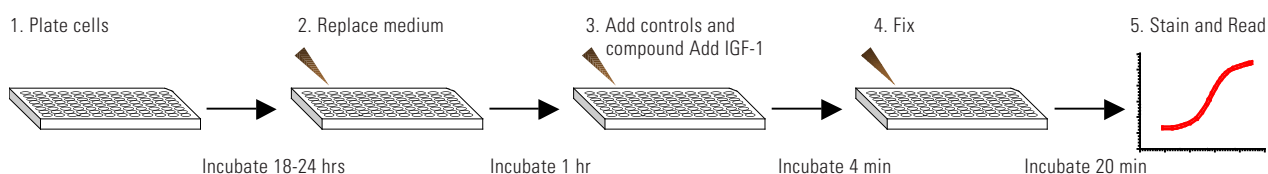
**Figure 2. Concentration response curve in the Akt3 antagonist assay.** Wortmannin concentration response curve in the Akt3 antagonist Redistribution assay stimulated by 100 nM IGF-1 (n=16). The EC<sub>50</sub> of wortmannin is 35 nM. Concentration response was measured in 9 point half log dilution 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 Celloomics ArrayScan V<sup>TI</sup> Reader and the CytoCellMemTrans.V2 BioApplication. % activity was calculated relative to the positive (300 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 Akt3 translocation event.



**Figure 4.** The Akt3 Redistribution assay is very easy and fast to perform.

## Thermo Scientific Akt3 Redistribution<sup>®</sup> Assay

### Assay Details

Recombinant CHOHR cells stably expressing human Akt3 fused to the N-terminus of enhanced green fluorescent protein (EGFP). The Akt3 Redistribution assay monitors translocation of an EGFP-human Akt3 fusion protein from the cytoplasm to the plasma membrane. Insulin-like growth factor-I (IGF-1) is used as reference agonist. Test compounds are assayed for their ability to inhibit IGF-1-stimulated membrane translocation of Akt3. The PI3K inhibitor wortmannin [1, 2] is used as reference antagonist. Compounds inhibiting IGF-1-induced translocation of Akt3 may interfere directly with Akt3 translocation (*e.g.* via PH-domain binding) or act upstream of Akt3. For further profiling of test compounds identified to interfere with membrane translocation of Akt3 analyzes of isoform selectivity can be performed by using the Akt1 and Akt2 Redistribution assays (see related products).

The Akt3 assay is validated with an average  $Z' = 0.58 \pm 0.08$ , suitable for both screening and profiling applications.

### Imaging

The translocation of Akt3-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 Akt3 Redistribution assay is the translocation of Akt3-EGFP 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>®</sup>

This assay has been validated on the Cellomics ArrayScan V<sup>®</sup> 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\_%MemColoc. 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 ColocalizationV3.

### Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
012_01	Akt3 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 domain Redistribution Assay	CHO	•	•	•
085_01	Akt1 Redistribution Assay	CHO	•	•	
011_02	Akt2 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	•		
8407201	Cellomics FOXO1A Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8404101	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			
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. Acaro A. & Wymann MP. *Biochem J.* 296, 297-301, 1993.
2. Burgering BM. & Coffey PJ. *Nature* 376, 599-602, 1995.

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