

Thermo Scientific Foxo4/AFX 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.

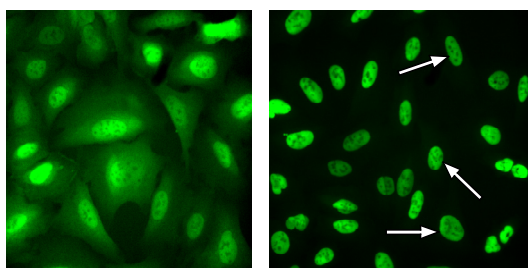


Figure 1. Translocation of Foxo4-EGFP. Cells were treated with 150 nM wortmannin for 1 hr (right panel) or untreated (DMSO control, left panel). Arrows indicate the nuclear localization detected by the image analysis algorithm.

Thermo Scientific Foxo4/AFX Redistribution Assay

Foxo4/AFX was first described as acute-lymphocytic-leukaemia-1 fused gene from chromosome X and is part of the Forkhead transcription factor family, which consists of Foxo1, Foxo3a, and Foxo4 (FKHR, FKHL1, and AFX, respectively). Forkhead transcription factors function as key regulators of insulin signaling, cell cycle progression and apoptosis downstream of phosphoinositide 3-kinase (PI3K). They act as classical transcriptional activators and share DNA-binding specificity to a core consensus site called the Forkhead-responsive element. Target genes include pro-apoptotic proteins (*e.g.* Fas Ligand and Bim) and cell cycle inhibitors (*e.g.* p27, p130 and GADD45). In growing cells, Forkheads are kept inactive through Akt-mediated phosphorylation of three conserved serines and threonines. Phosphorylation of these residues causes binding to 14-3-3 proteins in the nucleus followed by nuclear export and cytoplasmic retention. Nuclear export of Forkhead proteins is dependent on the classical NES/Crm1 pathway [1]. Inactive

Foxo4/AFX is cytoplasmic but is rapidly imported to the nucleus upon inactivation of the PI3K/Akt pathway. Foxo4/AFX nuclear translocation can be promoted by the PI3K inhibitor wortmannin [2, 3].

Features

- Designed to assay compounds for their ability to modulate nuclear translocation of Foxo4/AFX
- 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

Wortmannin concentration response curve in the Foxo4/AFX Redistribution assay

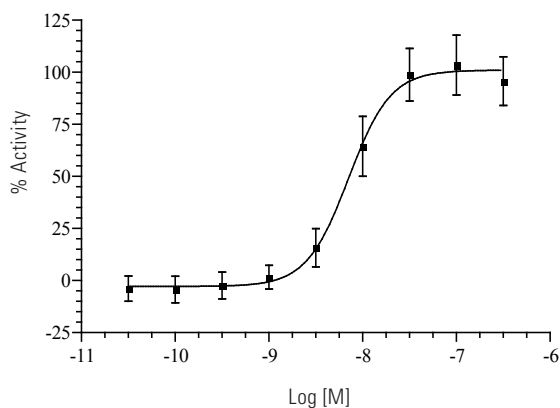


Figure 2. Wortmannin concentration response in the Foxo4/AFX Redistribution assay. Concentration response was measured in 9 point half log dilution series (n = 16). Cells were incubated with wortmannin for 1 hr. Cells were then fixed and the nucleus to cytoplasm translocation was measured using the Cellomics ArrayScan V^{TI} Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (150 nM wortmannin) and negative control (0.25% DMSO). The EC₅₀ of wortmannin is ~7nM.

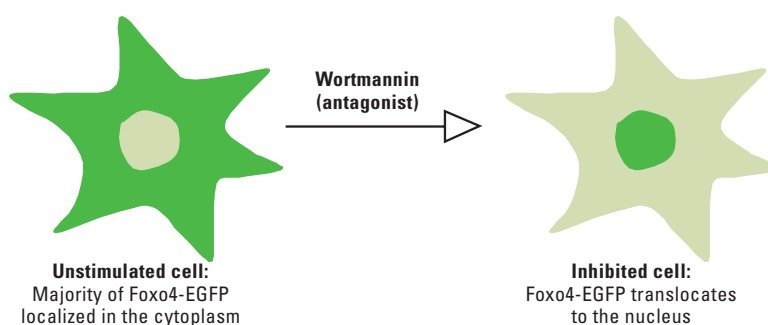


Figure 3. Illustration of the Foxo4/AFX domain translocation event.

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Assay Details

Recombinant U2OS cells stably expressing human Foxo4/AFX fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to test for Foxo4/AFX activation by monitoring the translocation of a Foxo4-EGFP fusion protein from the cytoplasm to the nucleus. Wortmannin is used as a reference compound and test compounds are assayed for their ability to induce nuclear accumulation of Foxo4/AFX, which indicates that compounds are either directly interfering with Foxo4/AFX export or acting upstream of Foxo4/AFX by interfering with the PI3K/Akt pathway. The Foxo4/AFX assay is validated with an average $Z' = 0.58 \pm 0.1$, suitable for both screening and profiling applications.

Imaging

The translocation of Foxo4-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 5-10x objective or higher magnification.

The primary output in the Foxo4/AFX Redistribution assay is the translocation from cytoplasm to nucleus of Foxo4-EGFP. The data analysis should therefore report an output relating to the GFP fluorescence intensities in the nucleus and the cytoplasm.

Imaging on Thermo Scientific Cellomics ArrayScan V^{TI}

This assay has been validated on the Cellomics Arrayscan V^{TI} using a 10x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the Redistribution V3 BioApplication. The output used was MEAN_CircRingAvgIntenRatioLog (Log of the ratio of average fluorescence intensities of nucleus and cytoplasm (well average)). 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 Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.

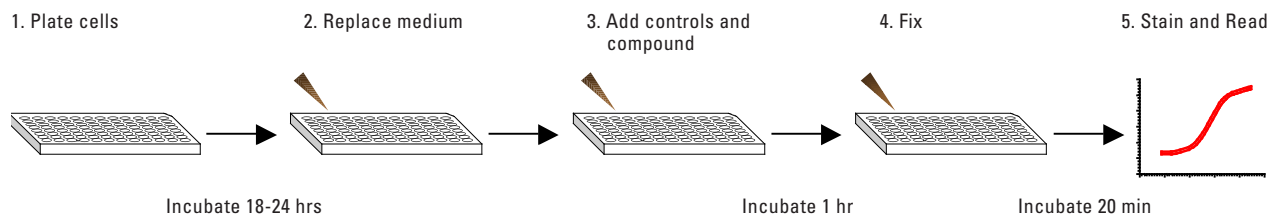


Figure 4. The Foxo4/AFX Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
090_01	Foxo4/AFX Redistribution Assay	U2OS	•	•	

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
008_01	FKHR (FOXO1) Redistribution Assay	U2OS	•	•	•
009_02	FKHRL1 (FOXO3a) Redistribution Assay	U2OS	•	•	•
085_01	Akt1 Redistribution Assay	CHO	•	•	
011_02	Akt2 Redistribution Assay	CHO	•	•	•
012_01	Akt3 Redistribution Assay	CHO	•	•	
062_01	REV Redistribution Assay	U2OS	•	•	
8404601	Cellomics Cell Cycle I HCS Reagent Kit	Antibody- and dye-based reagent kit			
8401101	Cellomics BrdU and Ki67 Cell Proliferation HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0500011	Cellomics Mitotic Index: Phospho-Histone H3 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 [®]	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

References

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

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