

Thermo Scientific FKHRL1 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.

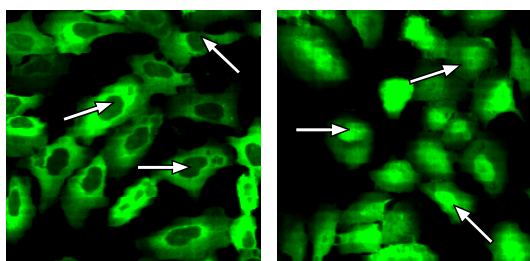


Figure 1. Translocation of FKHRL1-EGFP in response to wortmannin. Cells were treated with 0.25% DMSO (left panel) or 300 nM wortmannin (right panel). Arrows indicate cytoplasm to nucleus translocation detected by the image analysis algorithm.

Thermo Scientific FKHRL1 Redistribution Assay

Forkhead proteins comprise a highly conserved family of transcription factors named after the original forkhead gene in *Drosophila*. Forkhead transcription factor family members (FKHR, FKHRL1 and AFX) are known to control the expression of genes encoding proteins essential for insulin, apoptosis (e.g. Fas Ligand and Bim) and cell cycle (e.g. p27, p130 and GADD45) signaling. The activity of FKHRL1 is regulated via its phosphorylation by the protein kinase Akt that is part of the phosphoinositide 3-kinase (PI3K) signaling pathway. Phosphorylated FKHRL1 is sequestered in its inactive form within the cytosol by the so-called 14-3-3 protein. Unphosphorylated and active FKHRL1 resides in the nucleus. Furthermore, cellular localization of forkhead proteins is also dependent on the classical nuclear

export sequence (NES)/Crm1 pathway [1]. Wortmannin inhibits PI3K signalling and hereby hinders FKHRL1 phosphorylation and cytoplasmic sequestering, eventually resulting in nuclear accumulation of FKHRL1 [2, 3]. In this assay wortmannin is used as reference compound. Test compounds are assayed for their ability to induce nuclear accumulation of FKHRL1.

Features

- Designed to assay compounds for their ability to modulate nuclear translocation of FKHRL1 (FOXO3)
- 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

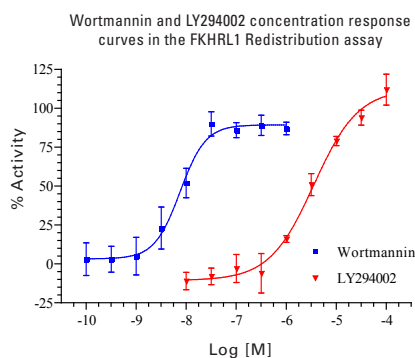


Figure 2. Wortmannin and LY294002 concentration response curves in the FKHRL1 Redistribution assay. Concentration response was measured in 9 point half log dilution series of wortmannin and LY294002. Cells were incubated with compound for 60 min. Cells were then fixed and the nucleus to cytoplasm translocation was measured using image analysis. % activity was calculated relative to the positive (300 nM wortmannin) and negative control (0.25% DMSO). The EC₅₀ of wortmannin is approximately 7.5 nM and the EC₅₀ of LY294002 is approximately 3.6 μM.

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

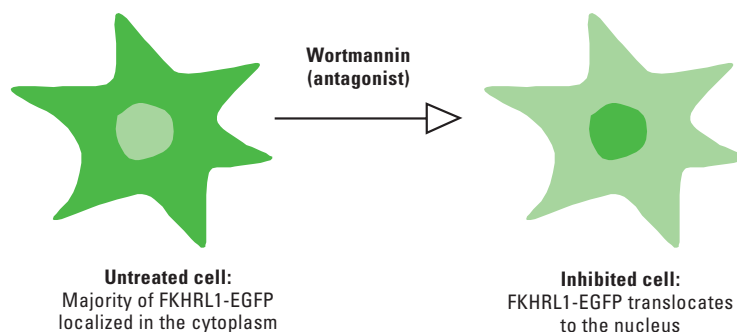


Figure 3. Illustration of the FKHL1 translocation event.

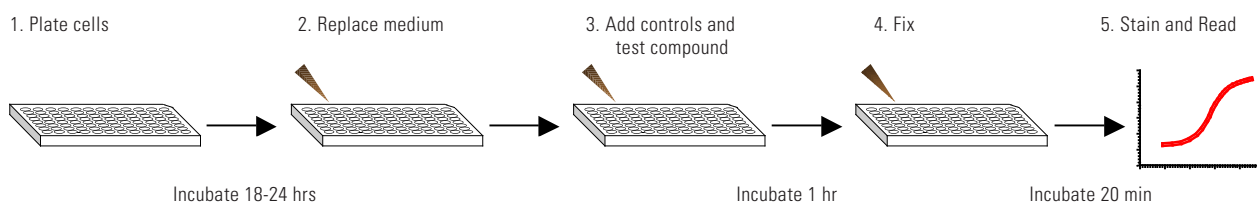


Figure 4. The FKHL1 Redistribution assay is very easy and fast to perform.

Thermo Scientific FKHL1 Redistribution® Assay

Assay Details

Recombinant U2OS cells stably expressing human FKHL1 (FOXO3) fused to the N-terminus of enhanced green fluorescent protein (EGFP). Test compounds causing accumulation of FKHL1 in the nucleus may interfere directly with FKHL1 import, act upstream of FKHL1 interfering with the PI3K/Akt1 signaling pathway, or may be general nuclear import activators/nuclear export inhibitors. For further profiling of test compounds identified as positive in the FKHL1 assay, isoform selectivity can be determined by using the FKHL1 and AFX Redistribution assays. Compounds acting upstream of Akt1 within the PI3K signaling pathway can be identified using the Akt Redistribution assays, and general export inhibitor characteristics of compounds can be determined using the Rev1 Redistribution assay (for further details - see related assays). The FKHL1 assay is validated with an average $Z' = 0.59 \pm 0.07$, suitable for both screening and profiling applications.

Imaging

The translocation of FKHL1-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 FKHL1 Redistribution assay is the translocation from cytoplasm to nucleus of FKHL1-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™

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 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 150 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2 and ColocalizationV3.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
009_02	FKHRL1 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	•	•	•
090_01	AFX/Foxo4 Redistribution Assay	U2OS	•	•	
085_01	Akt1 Redistribution Assay	CHO	•	•	
011_02	Akt2 Redistribution Assay	CHO	•	•	•
012_01	Akt3 Redistribution Assay	CHO	•	•	
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			
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 ^{TI}	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

References

1. Burgering BMT. & Kops GJPL. *Trends Biochem. Sci.* 27, 352-360, 2002.
2. Acaro A. & Wymann MP. *Biochem J.* 296, 297-301, 1993.
3. Burgering BM. & Coffey PJ. *Nature* 376, 599-602, 1995.

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