

Thermo Scientific FKHR 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.

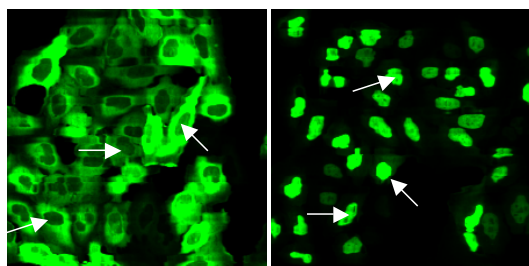


Figure 1. Translocation of FKHR-EGFP in response to wortmannin. Cells were treated with 0.25% DMSO (left panel) or 300 nM wortmannin (right panel). Arrows indicate cytoplasm to nucleus translocation detected by the image analysis algorithm.

Thermo Scientific FKHR 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, FKHL1 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 FKHR is regulated via its phosphorylation by the protein kinase Akt that is part of the phosphoinositide 3-kinase (PI3K) signaling pathway. Phosphorylated FKHR is sequestered in its inactive form within the cytosol by the so-called 14-3-3 protein. Un-phosphorylated and active FKHR reside 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 FKHR phosphorylation and cytoplasmic sequestering, eventually resulting in nuclear accumulation of FKHR [2, 3].

Features

- Designed to assay compounds for their ability to modulate nuclear translocation of FKHR (FOXO1)
- 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

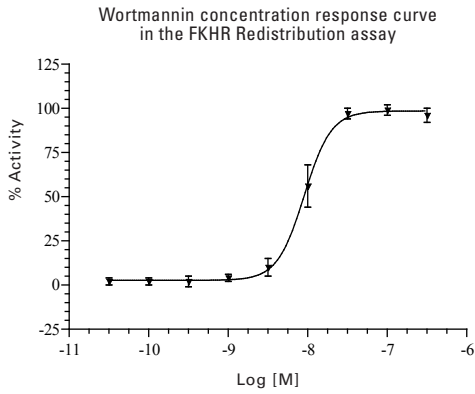


Figure 2. Wortmannin concentration response curve in the FKHR Redistribution assay. Concentration response was measured in 9 point half log dilution series of wortmannin. Cells were incubated with wortmannin for 60 min. Cells were then fixed and the nucleus to cytoplasm translocation was measured using the Celloomics ArrayScan VTM Reader and the RedistributionV3 BioApplication. % activity was calculated relative to the positive (150 nM wortmannin) and negative control (0.25% DMSO). The EC₅₀ of wortmannin is approximately 9 nM.

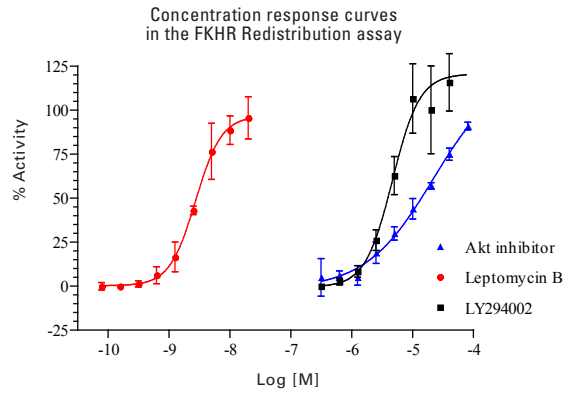


Figure 3. Concentration response curves in the FKHR Redistribution assay. Concentration response was measured in 9 point 2x dilution series of Leptomycin B (nuclear export inhibitor), LY294009 (PI3K inhibitor) and an Akt inhibitor [4]. Cells were incubated with test compound for 60 min. Cells were then fixed and the nucleus to cytoplasm translocation was measured using image analysis. % activity was calculated relative to the positive (150 nM wortmannin) and negative control (0.25% DMSO). The EC₅₀ values are: Leptomycin EC₅₀= 2.7 nM, LY294002 EC₅₀=4.7 μM and Akt inhibitor EC₅₀=22.

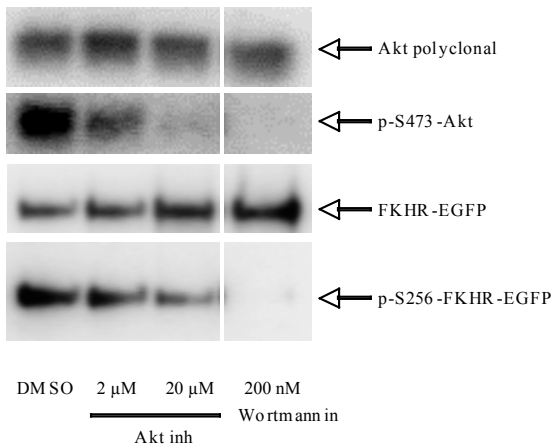


Figure 4. Western blot showing regulation of FKHR Redistribution cells. FKHR Redistribution cells were incubated with DMSO, wortmannin or an Akt inhibitor [4] for 60 min. Cells were then lysed and cell extracts were run on western blot. *Akt polyclonal* and *FKHR-EGFP* indicate the total amount of Akt1-3 and FKHR-EGFP in the assay cell line, respectively. *P-S473-Akt* indicates the amount of Akt that is activated by S473 phosphorylation. This activation is fully inhibited by wortmannin and the Akt inhibitor. *P-S256-FKHR-EGFP* indicates the amount of FKHR-EGFP that is phosphorylated on S256 (Akt site). FKHR phosphorylation is fully inhibited by wortmannin and partly inhibited by the Akt inhibitor.

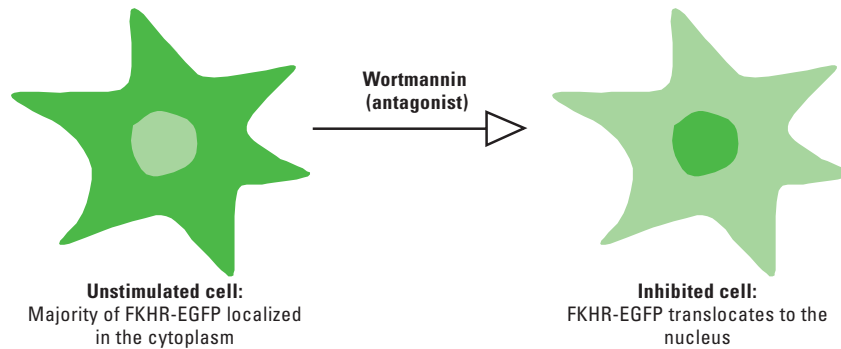


Figure 5: Illustration of the FKHR translocation.

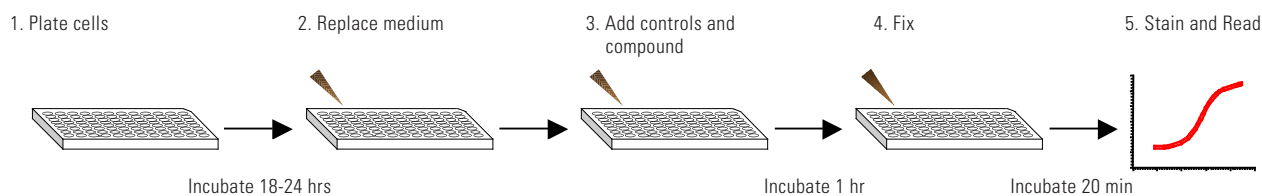


Figure 6: The FKHR Redistribution assay is very easy and fast to perform.

Thermo Scientific FKHR Redistribution® Assay

Assay Details

Recombinant U2OS cells stably expressing human FKHR/FOXO1 fused to the N-terminus of enhanced green fluorescent protein (EGFP). In this assay wortmannin is used as reference compound. Test compounds are assayed for their ability to induce nuclear accumulation of FKHR. Test compounds causing accumulation of FKHR in the nucleus may interfere directly with FKHR import, act upstream of FKHR 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 FKHR assay, isoform selectivity can be determined by using the FKHL1 Redistribution assay [4]. Possible effect of positive test compounds upstream of Akt1, within the PI3K signaling pathway, can be determined using the Akt1 Redistribution assay [5]. General export inhibitor characteristics of compounds can be performed using the Rev1 Redistribution assay [6]. Finally, the FKHR Redistribution assay can be used also to detect compounds acting as general nuclear export inhibitors; in this case the nuclear export inhibitors Leptomycin B or Ratjadone A is used as reference compound. The FKHR assay is validated with an average with an average $Z' = 0.66 \pm 0.05$, suitable for both screening and profiling applications.

Imaging

The translocation of FKHR-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 FKHR Redistribution assay is the translocation from cytoplasm to nucleus of FKHR-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 developed 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 100 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
008_01	FKHR 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
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

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3. Burgering BM. & Coffey PJ. *Nature* 376, 599-602, 1995.
4. Barnett, SF et al. *Curr Top Med Chem* 5, 109-125, 2005.

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