

Thermo Scientific Hexokinase II 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.

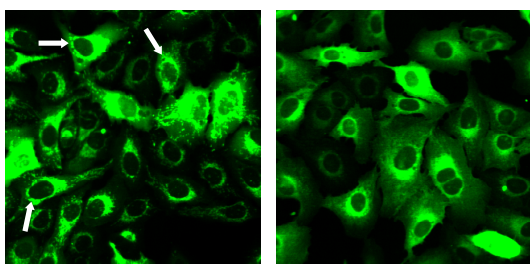


Figure 1. Translocation of HKII-EGFP in response to clotrimazole. Cells were treated with 100 μ M clotrimazole (right panel) or untreated (DMSO control, left panel). Arrows indicate localization of HKII-EGFP in mitochondria detected by the image analysis algorithm.

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Hexokinase II (HKII) mediates the first step of glycolysis, by phosphorylation of glucose to glucose 6-phosphate. It has been known for many years that tumor cells to a large extent depend on glycolysis to produce ATP. This is partly due to overexpression of HKII, which is predominantly localized to mitochondria through binding to the voltage-dependent anion channel (VDAC). This localization enables HKII to get preferential access to mitochondrial ATP and thereby create an increase in the rate of glycolysis, known as aerobic glycolysis. In addition, binding of HKII to VDAC is believed to prevent binding of Bax to mitochondria and thereby block release of cytochrome C, resulting in decreased sensitivity to apoptosis [1, 2]. Thus, compounds that cause dissociation of HKII from mitochondria have potential to act directly as anti-cancer agents or to potentiate the effect of chemotherapeutic agents and thereby be useful in anti-cancer treatment.

Features

- Designed to assay compounds for their ability to release Hexokinase II from mitochondria
- 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

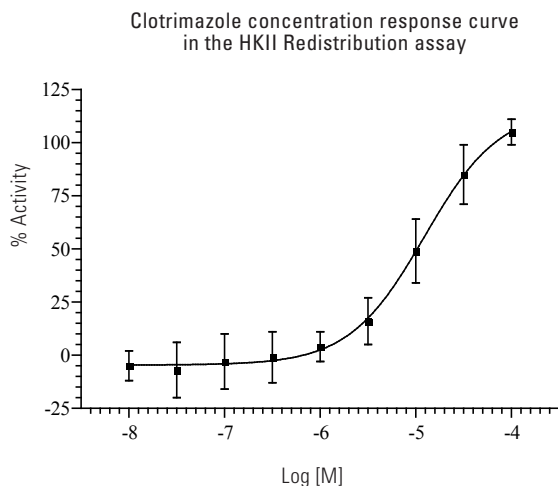


Figure 2: Clotrimazole concentration response curve in the HKII Redistribution assay. Concentration response was measured in 9 point half log dilution series of clotrimazole ($n = 8$). Cells were then fixed and the mitochondria to cytoplasm translocation was measured using the Cellomics ArrayScan V^{TI} Reader and the SpotDetector V3 BioApplication. % activity was calculated relative to the positive (100 μ M clotrimazole) and negative control (0.25% DMSO). The EC₅₀ value of clotrimazole in the assay is approximately 15 μ M.

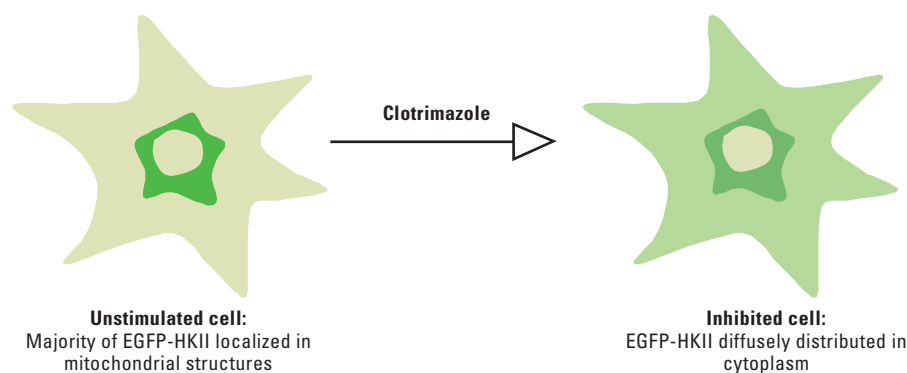


Figure 3. Illustration of the EGFP-HKII translocation.

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Assay Details

Recombinant U2OS cells stably expressing human Hexokinase II (HKII) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The HKII Redistribution assay is designed to screen for agents that induce translocation of EGFP-HKII from mitochondria to a diffuse localization in the cytoplasm. Clotrimazole is used as a reference compound, since it has been demonstrated to dissociate HKII from mitochondria [3]. The Hexokinase II assay is validated with an average $Z' = 0.46 \pm 0.16$, suitable profiling applications.

Imaging

The translocation of EGFP-Hexokinase 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 10-20x objective or higher magnification. The primary output in the Hexokinase Redistribution assay is the translocation of EGFP-Hexokinase from punctate localization (mitochondria) to a diffuse localization in the cytoplasm. The data analysis should therefore report an output that corresponds to number, area, or intensity of EGFP-Hexokinase spots in 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), High Resolution images, XF100 filter sets for Hoechst and FITC, and the SpotDetectorV3 BioApplication. The output parameter used was SpotTotalAreaPerObject. 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 CompartmentalAnalysisV2 and ColocalizationV3.

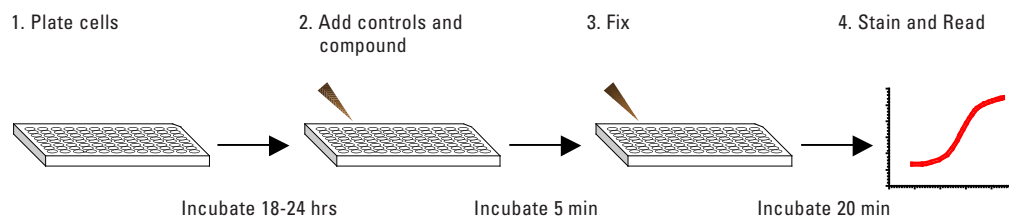


Figure 4. The Hexokinase II Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
050_01	Hexokinase II 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
042_01	Rad51 Redistribution Assay	SW480	•	•	•
8405601	Cellomics Cytochrome C Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
8402201	Cellomics Caspase 3 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8402301	Cellomics Caspase 9 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. Pastorino, J.G. *Curr Med Chem.* 10(16):1535-51, 2003.
2. Majewski, N. et al., *Mol Cell.* 16(5):819-30, 2004.
3. Penso, J. et al., *Eur. J. Pharmacol.* 342(1):113-7, 1998.

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