

Thermo Scientific HIF-1 α 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.

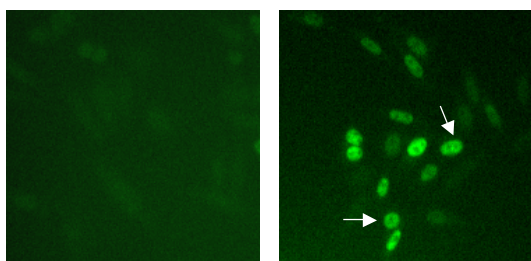


Figure 1. Accumulation of EGFP-HIF-1 α . Cells were treated with 0.25% DMSO (control, left panel) or 100 μ M 2,2'-dipyridyl (right panel). Arrows indicate 2,2'-dipyridyl-induced accumulation of EGFP-HIF-1 α detected by the image analysis algorithm.

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HIF-1 is a heterodimeric transcription factor consisting of HIF-1 α and β subunits that permits activation of genes essential to cellular adaptation to low oxygen conditions (i.e. hypoxia). HIF-1 β is constitutively expressed, whereas the expression of HIF-1 α is maintained at low levels under normal oxic conditions (i.e. normoxia).

During normoxia HIF-1 α is hydroxylated on prolyl residues by the O₂-dependent HIF1 α prolyl-hydroxylase (PDH), which targets it for degradation mediated by the E3 ubiquitin ligase activity of the von-Hippel-Lindau (VHL) tumor suppressor protein. Under hypoxic conditions, O₂ becomes rate limiting for prolyl hydroxylation, resulting in decreased ubiquitination of HIF-1 α by VHL. This leads to accumulation of HIF-1 α in the nucleus where it regulates a number of target genes

involved in adaptation to hypoxic conditions. Since tumor cells are more hypoxic than normal cells and hypoxia is associated with poor prognosis and resistance to treatment, HIF-1 α is considered to be a promising therapeutic target to kill tumor cells. Thus, manipulating the HIF-1 pathway has potential in treatment of several diseases including cancer and ischemia [1-3].

Features

- Designed to assay compounds for their ability to induce nuclear accumulation of HIF-1 α
- 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

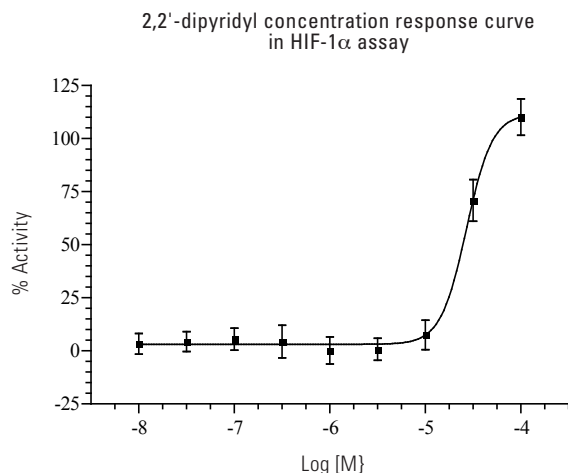


Figure 2. Concentration response curve in the HIF-1 α assay (n = 8). Concentration response was measured in 9 point half log dilution series. Cells were incubated with test compound for 3 hours. Cells were then fixed and accumulation was measured using the Cellomics ArrayScan V^{TI} Reader and the RedistributionV3 BioApplication. % activity was calculated relative to the positive (100 μ M 2,2'-dipyridyl) and negative control (0.25% DMSO). The EC₅₀ value of 2,2'-dipyridyl is approximately 27 μ M.

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Assay Details

Recombinant CHOhr cells stably expressing human HIF-1 α fused to the N-terminus of enhanced green fluorescent protein (EGFP). CHOhr cells are adherent epithelial cells derived from Chinese hamster ovary expressing human insulin receptor. The assay is set up to monitor accumulation of HIF-1 α in the nucleus in response to the chemical hypoxia-mimetic 2,2'-dipyridyl (DP). Iron chelators such as DP are thought to be hypoxia mimetics since they interfere with PDH and thereby stabilize HIF-1 α under normoxic conditions. DP is used as reference compound in the assay, and compounds are assayed for their ability to induce nuclear translocation of HIF-1 α . The HIF-1 α assay is validated with an average Z' = 0.61 \pm 0.12, suitable for both screening and profiling applications.

Imaging

The translocation of HIF-1 α 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 HIF-1 α Redistribution assay is the translocation from the cytoplasm to the nucleus. 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 parameter used was MEAN_CircAvgInten. The minimally acceptable number of cells used for image analysis in each well was set to 300 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2 and ColocalizationV3.

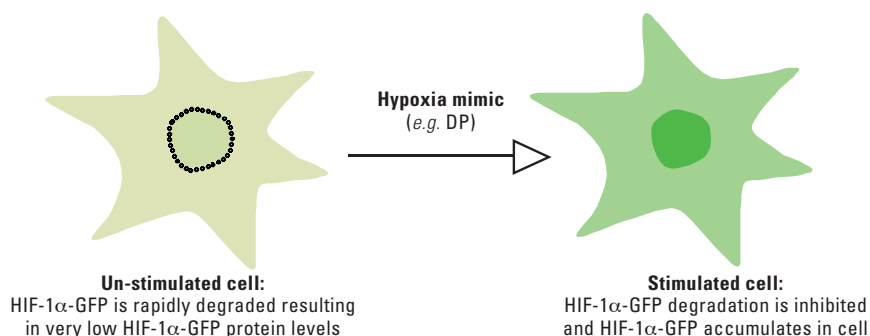


Figure 3. Illustration of the HIF-1 α translocation event.

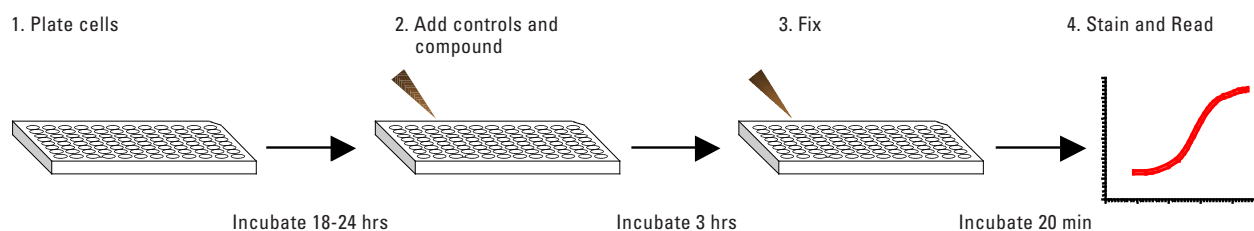


Figure 4. The HIF-1 α Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
066_01	HIF-1 α 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
066_02	HIF-1 α Redistribution Assay	U2OS	•	•	•
8401601	Cellomics HIF-1 alpha HCS Reagent Kit	Antibody- and dye-based reagent kit			
8401501	Cellomics Phospho-CREB HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100111	Cellomics NFAT-1 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

- Hewitson KS and Schofiels CJ. *Drug Discov Today.*, 9,704-711, 2004.
- Ke Q & Costa M., *Mol Pharmacol.* 70, 1469-80, 2006.
- Déry MC. et al. *Int J Biochem Cell Biol.*, 37,535-540, 2005.

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