abcam

Product datasheet

Human Hif1 alpha+ GLUT Hypoxia In Cell ELISA Kit (IR) ab125298

6 Images

Overview

Product name Human Hif1 alpha+ GLUT Hypoxia In Cell ELISA Kit (IR)

Detection method IR

Precision

				Intra-assay
Sample	**n**	**Mean**	**SD**	**CV%**
HeLa cells				< 8%

Sample type Adherent cells, Suspension cells

Assay type Cell-based (quantitative)

Assay duration Multiple steps standard assay

Species reactivity **Reacts with:** Human
Does not react with: Mouse, Rat

Product overview Human Hif1 alpha+ GLUT Hypoxia (ab125298) is an In-Cell ELISA (ICE) assay kit that uses quantitative immunocytochemistry to measure HIF1 alpha and GLUT1 protein levels in cultured cells. Cells are fixed in a microplate and targets of interest are detected with highly specific, well-characterized antibodies. Relative protein levels are quantified using IRDye®-labeled Secondary Antibodies and IR imaging using a LI-COR® Odyssey® or Aerius® system.

Hypoxia and the cellular response to hypoxic environment is a central topic in studies of metabolism, cancer progression and development and stem cells. A key player is the transcription factor HIF1 alpha (hypoxia inducible factor 1 alpha) which is stabilized at the protein level in response to decreased oxygen tension. HIF1 alpha then promotes transcription of a number of factors that alters cellular physiology. This Hypoxia ICE assay kit provides duplexed measurements of the transcription factor HIF1 alpha and the HIF1A responsive protein GLUT1. "

HIF1 alpha is a constitutively expressed transcription factor that is degraded under normal oxygen tensions but stabilized (at the protein level) when oxygen is limiting (hypoxia). Under hypoxic conditions, stabilized HIF1A promotes the transcription of a host of genes that enable the cell to adapt to the lack of oxygen. A key aspect of the hypoxic response is the switch from aerobic respiration to anaerobic glycolysis and many of the HIF1 alpha responsive genes encode proteins that promote glycolysis and/or inhibit oxidative phosphorylation. Stabilization of the HIF1 alpha protein levels can be detected quickly after the onset of hypoxia, whereas

changes in the levels of HIF1 alpha responsive proteins take longer to manifest. The anti-HIF1 alpha antibody used in this assay is specific for Human HIF1 alpha protein.

GLUT1 is a widely expressed cell membrane glucose transporter and is responsible for basal glucose uptake. GLUT1 can transport a range of aldolases including pentoses and hexoses. Stabilization of the HIF1 alpha transcription factor directly leads to increased transcription of GLUT1. In turn, increased expression of GLUT1 in response to hypoxia is thought to provide additional sugars for anaerobic glycolysis. The gene name for GLUT1 is Solute carrier family 2, facilitated glucose transporter member 1 (SLC2A1). The anti-GLUT1 antibody in this assay kit is reactive with Human, Mouse and Rat GLUT1.

In-Cell ELISA (ICE) technology is used to perform quantitative immunocytochemistry of cultured cells with a near-infrared fluorescent dye-labeled detector antibody. The technique generates quantitative data with specificity similar to Western blotting, but with much greater quantitative precision and higher throughput due to the greater dynamic range and linearity of direct fluorescence detection and the ability to run 96 samples in parallel. This method rapidly fixes the cells in situ, stabilizing the in vivo levels of proteins, and thus essentially eliminates changes during sample handling, such as preparation of protein extracts. Finally, the HIF1 alpha and GLUT1 signals can all be normalized to cell amount, measured by the provided Janus Green whole cell stain, to further increase the assay precision.

Plates are available in our ICE (In-Cell ELISA) Support Pack (ab111542) which can be bought seperately.

Notes

Upon receipt spin down the contents of the IRDye®-labeled Secondary Antibody tube and protect from light. Store all components upright at 4°C. This kit is stable for at least 6 months from receipt.

Tested applications

Suitable for: In-Cell ELISA

Platform

Microplate

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
1000X IRDye-labeled Secondary Antibodies	1 x 24µl

Components	1 x 96 tests
100X GLUT1 Primary Antibody (Rabbit)	1 x 120µl
100X HIF1alpha Primary Antibody (Mouse)	1 x 120µl
100X Triton X-100	1 x 0.5ml
10X Blocking Solution	1 x 10µl
10X Phosphate Buffered Saline	1 x 100ml
400X Tween-20	1 x 2ml
Janus Green Stain	1 x 17ml

Cellular localization Hif1: Nucleus. Chromosome. GLUT: Cell Membrane.

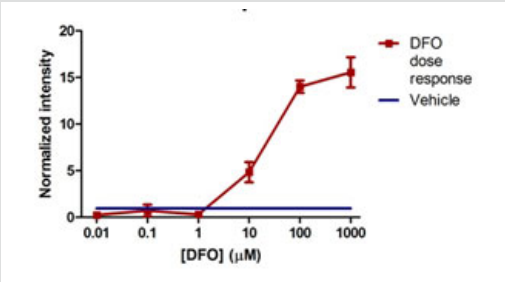
Applications

Our Abpromise guarantee covers the use of **ab125298** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

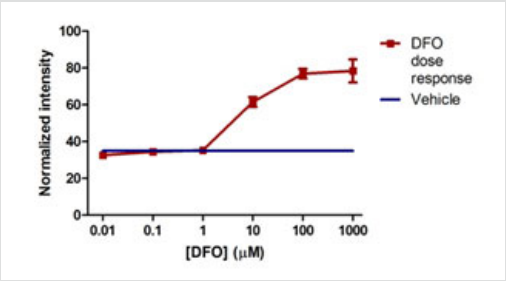
Application	Abreviews	Notes
In-Cell ELISA		Use at an assay dependent concentration.

Images



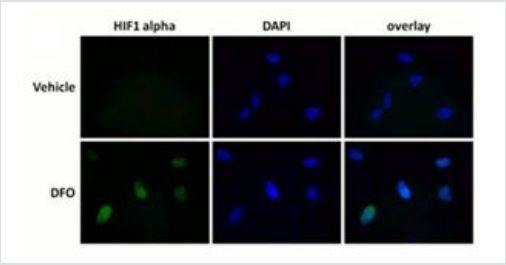
In-Cell ELISA - Hif1 + GLUT Hypoxia Human In Cell ELISA Kit (ab125298)

Sample experiment using ab125298 on HeLa cells treated with a titration of DFO. HeLa cells were seeded to an amine coated 96-well microplate and the following day treated with a titration of DFO. After 24h of DFO exposure, the cells were fixed and stained as described in the protocol and the normalized data is presented here +/- SD (as described in the protocol and data analysis sections). HIF1 alpha results show DFO concentrations >10µM induce HIF1A protein levels in a dose dependent manner.



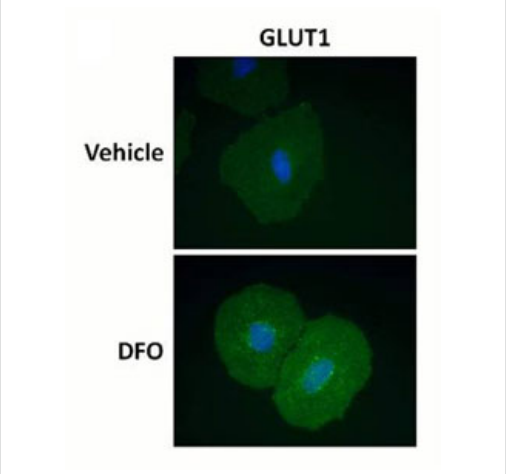
In-Cell ELISA - Hif1 + GLUT Hypoxia Human In Cell ELISA Kit (ab125298)

Sample experiment using ab125298 on HeLa cells treated with a titration of DFO. HeLa cells were seeded to an amine coated 96-well microplate and the following day treated with a titration of DFO. After 24h of DFO exposure, the cells were fixed and stained as described in the protocol and the normalized data is presented here +/- SD (as described in the protocol and data analysis sections). GLUT1 levels are increased with >10µM DFO.



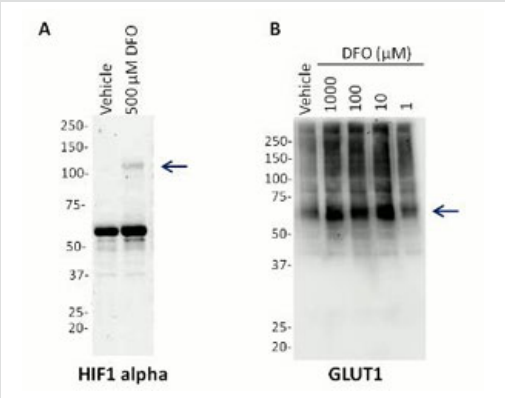
Immunocytochemistry/ Immunofluorescence - Hif1 + GLUT Hypoxia Human In Cell ELISA Kit (ab125298)

Antibody specificity demonstrated by immunocytochemistry. Primary antibodies used in this assay kit were validated by staining HeLa cells +/- treatment with 1mM DFO (24h) and imaged by fluorescent microscopy. HIF1 alpha (ab1, 4µg/mL) staining is absent in untreated cells and induced by DFO treatment. HIF1 alpha localizes to the nucleus (as seen by co-localization with the DNA stain DAPI) as expected.



Immunocytochemistry/ Immunofluorescence - Hif1 + GLUT Hypoxia Human In Cell ELISA Kit (ab125298)

Antibody specificity demonstrated by immunocytochemistry. Primary antibodies used in this assay kit were validated by staining HeLa cells +/- treatment with 1mM DFO (24h) and imaged by fluorescent microscopy. GLUT1 staining (ab115730, diluted 1:5000) labels the cell membrane and the fluorescent intensity increases with DFO treatment.



Western blot - Hif1 + GLUT Hypoxia Human In Cell ELISA Kit (ab125298)

Antibody specificity demonstrated by Western Blot. Primary antibodies used in this assay kit were validated by Western Blot using HeLa cell lysates that had been treated with a dose titration of DFO as indicated. (A) The HIF1 alpha (ab1) band (indicated by arrow) is absent in untreated cells and induced by DFO. In the Western Blot assay, the HIF1 alpha antibody has a prominent ~60kDa background band; however background signal is absent in ICE and immunocytochemistry application (Fig. 1 and Fig. 2). (B) Similarly, GLUT1 levels are increased by DFO treatment in a dose-dependent manner. GLUT1 can be phophorylated and glycosylated which likely contributes to the “smeary” bands on the Western Blot membrane.

Coefficient of variation

		replicates	HIF1A	GLUT1
Vehicle		6	5%	2%
DFO (µM) dose response	1000	3	2%	2%
	100	3	2%	2%
	10	3	4%	1%
	1	3	6%	1%
	0.1	3	3%	2%
	0.01	3	2%	1%

In-Cell ELISA - Hif1 + GLUT Hypoxia Human In Cell ELISA Kit (ab125298)

Coefficient of variation for the experiment described in Figure 1.

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