abcam

Product datasheet

Human Hif1a + BNIP3 Hypoxia In Cell ELISA Kit (IR) ab129733

6 Images

Overview

Product name	Human Hif1a + BNIP3 Hypoxia In Cell ELISA Kit (IR)
Detection method	IR
Sample type	Adherent cells, Suspension cells
Assay type	Cell-based (quantitative)
Assay duration	Multiple steps standard assay
Species reactivity	**Reacts with:** Human
Product overview	ab129733 is an In-Cell ELISA (ICE) assay kit that uses quantitative immunocytochemistry to measure HIF1 alpha and BNIP3 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 BNIP3. Plates are available in our ICE (In-Cell ELISA) Support Pack (**ab111542**) which can be bought seperately.
Notes	Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.
Platform	Microplate

Properties

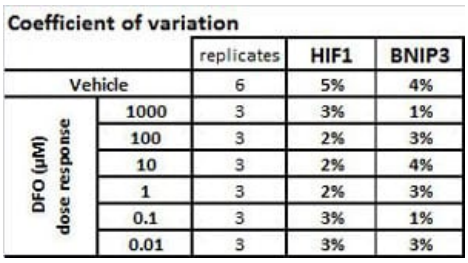
Storage instructions Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
1000X IRDye-labeled Secondary Antibodies	1 x 24µl
100X BNIP3 Primary Antibody (Mouse)	1 x 120µl
100X HIF1alpha Primary Antibody (Rabbit)	1 x 120µl
100X Triton X-100	1 x 0.5ml
10X Blocking Solution	1 x 10ml
10X Phosphate Buffered Saline	1 x 100ml
400X Tween-20	1 x 2ml
1X Janus Green Stain	1 x 17ml

Relevance The gene encodes the alpha subunit of transcription factor hypoxia-inducible factor-1 (HIF-1), which is a heterodimer composed of an alpha and a beta subunit. HIF-1 functions as a master regulator of cellular and systemic homeostatic response to hypoxia by activating transcription of many genes, including those involved in energy metabolism, angiogenesis, apoptosis, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia. HIF-1 thus plays an essential role in embryonic vascularization, tumor angiogenesis and pathophysiology of ischemic disease. Alternatively spliced transcript variants encoding different isoforms have been identified for this gene. The gene is a member of the BCL2/adenovirus E1B 19 kd-interacting protein (BNIP) family. It interacts with the E1B 19 kDa protein, which protects cells from virally-induced cell death. The encoded protein also interacts with E1B 19 kDa-like sequences of BCL2, another apoptotic protector. This protein contains a BH3 domain and a transmembrane domain, which have been associated with pro-apoptotic function. The dimeric mitochondrial protein encoded by this gene is known to induce apoptosis, even in the presence of BCL2.

Cellular localization HIF1a: Cytoplasmic and Nuclear. Note: Cytoplasmic in normoxia, nuclear translocation in response to hypoxia. Colocalizes with SUMO1 in the nucleus, under hypoxia. BNIP3: Mitochondrion. Mitochondrion membrane; Single-pass membrane protein. Note: Coexpression with the EIB 19-kDa protein results in a shift in NIP3 localization pattern to the nuclear envelope. Colocalizes with ACAA2 in the mitochondria.

Images

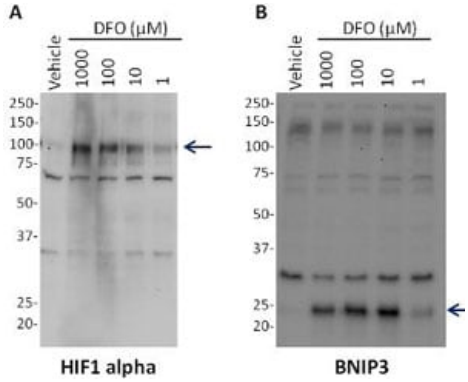


Coefficient of variation

		replicates	HIF1	BNIP3
Vehicle		6	5%	4%
DFO (µM) dose response	1000	3	3%	1%
	100	3	2%	3%
	10	3	2%	4%
	1	3	2%	3%
	0.1	3	3%	1%
	0.01	3	3%	3%

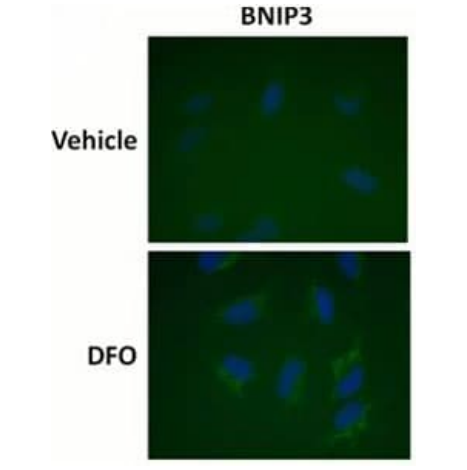
In-Cell ELISA - Hif1a + BNIP3 Hypoxia Human In Cell ELISA Kit (IR) (ab129733)

Coefficient of variation for the experiment described in Figure 1.



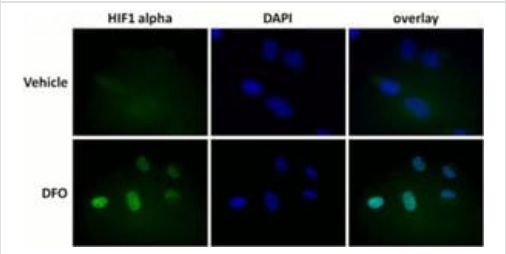
Western blot - Hif1a + BNIP3 Hypoxia Human In Cell ELISA Kit (IR) (ab129733)

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 band (indicated by arrow) is absent in untreated cells and induced by DFO. (B) Similarly, BNIP3 levels are increased by DFO treatment in a dose-dependent manner.



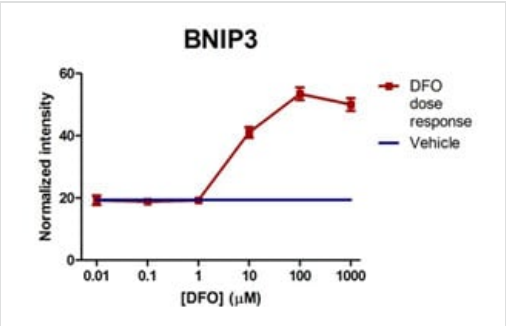
Immunocytochemistry/ Immunofluorescence - Hif1a + BNIP3 Hypoxia Human In Cell ELISA Kit (IR) (ab129733)

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. BNIP3 staining is nearly undetectable in untreated HeLa cells but is induced by DFO treatment. BNIP3 appears to has a mitochondrial staining pattern in the DFO-treated samples.



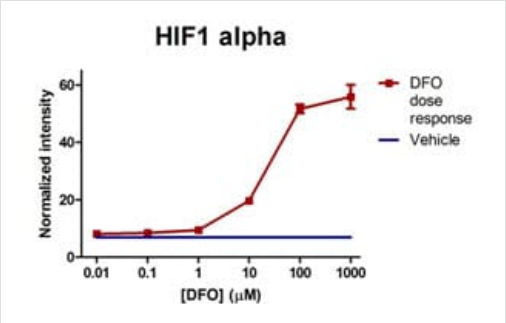
Immunocytochemistry/ Immunofluorescence - Hif1a + BNIP3 Hypoxia Human In Cell ELISA Kit (IR) (ab129733)

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 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.



In-Cell ELISA - Hif1a + BNIP3 Hypoxia Human In Cell ELISA Kit (IR) (ab129733)

Sample experiment using ab129733 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). BNIP3 levels are increased with DFO concentrations ≥10µM.



In-Cell ELISA - Hif1a + BNIP3 Hypoxia Human In Cell ELISA Kit (IR) (ab129733)

Sample experiment using ab129733 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.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you

- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors