HIF-1 alpha

Western Blot

https://www.abcam.com/protocols/general-western-blot-protocol

Target Overview in WB Application

HIF-1 alpha is stabilized only at O_2 concentration below 5%. Under normoxic conditions HIF-1 alpha has a short half-life and may be degraded within 5-8 minutes in both nuclear and cytoplasmic compartments. Therefore, **proper sample preparation** is critical to aiding WB success. If care hasn't been taken with sample preparation no bands may be seen on your blot.

Here are a few tips to help ensure the best results in WB:

Sample Preparation	•Add adequate **protease inhibitors** (or phosphatase inhibitors for proteins modified by phosphorylation) to avoid target protein degradation.
	•**Ultrasonicate** samples to enrich more target proteins.
	•Keep samples on **ice** during the whole WB process.
	•Perform a Bradford assay, a Lowry assay or a bicinchoninic acid (BCA) assay to **determine the protein concentration**.
Electrophoresis	•For large proteins (the MW of target protein >100 kDa), be sure to run samples in 8% or lower separating gel.
	•Load **at least 50µg** total protein per lane.
	•We strongly recommend the use of a **positive control lysate** when setting up a new experiment; this will give you immediate confidence in the protocol.
Transferring	•It is preferred to **add SDS** to a final concentration of 0.1% in the transfer buffer for large proteins.
	•Wash PVDF membrane to **remove methanol** completely.
	•To determine if the transfer is successful by visualization of proteins in membranes using **Ponceau S**.

You should pay attention to these notes to maximize the signal:

- ✓ **Hypoxic chambers** may be used to incubate samples overnight at low oxygen pressure to induce HIF-1 alpha levels.
- ✓ Cells should be lysed as quickly (within 2 mins) as possible if removed from hypoxia.
- ✓ Use **positive control samples** such as nuclear lysates of DFO or $CoCl_2$ treated cells.
- ✓ **Overnight incubation** at 4°C with the primary antibody can also help.
- ✓ The observed band size of HIF-1 alpha is not exactly as predicted 93 kDa in WB due to the different forms of HIF-1 alpha as blow:
  - **40-80 kDa** - degraded HIF-1 alpha
  - **110-130 kDa** - post translationally modified HIF-1 alpha
  - **~200 kDa** - heterodimer with HIF-1 beta

Protein Function	Functions as a master transcriptional regulator of the adaptive response to hypoxia. Under such conditions, it can activate over 40 genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia. SwissProt: Q16665
Expression	- Most cell lines and tissues (under hypoxic conditions) - Highest levels in kidney and heart - Overexpressed in majority of human cancers Under normoxic conditions, HIF-1 alpha is largely undetectable. Hypoxia needs to be induced in most cells and normal tissues.
Location	Cytoplasmic in normoxic conditions. Nuclear translocation in response to hypoxia. PMID: 9822602
Isoforms	- Isoform 1: 93 kDa (predicted) - Isoform 2: 83 kDa (predicted) - Isoform 3: 96 kDa (predicted) **The observed band size of HIF-1 alpha may not be the same as predicted MWs in WB due to the different forms of HIF-1 alpha.**
Modifications	Snitrosylation/Phosphorylation/Sumoylation/ Acetylation/Polyubiquitnation/Hydroxyalation **The observed band size of HIF-1 alpha may not be the same as predicted MWs in WB due to these modifications.**
Positive Controls	**WB**: Hypoxic samples such as **HeLa-DFO treated whole cell lysate** ab116322. For a stronger signal, **HeLa-DFO treated nuclear extracts** are recommended ab180880. The cell fractionation kit can also be purchased separately ab109719.
Negative Controls	Most normal tissues or cells, other than kidney or heart.
Treatments	$CoCl_2$ or DFO to induce hypoxia. PMID: 3217626 Protease inhibitors like MG132 can stabilize HIF-1 alpha. PMID: 19347037

References

Journal	abID	Application	Species	PMID
Nature Communications (2019)	ab1	WB	Mouse	31391533
Cell (2019)	ab179483	WB	Mouse	31708126
Science Advances (2019)	ab2185	WB	Mouse	31281892