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:**

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

**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
Nitrosylation/Phosphorylation/Sumoylation/Acetylation/Polyubiquitnation/Hydroxylation

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
**CoCl₂ or DFO to induce hypoxia.**  
**Protease inhibitors like MG132 can stabilize HIF-1 alpha.**  
**PMID:** 19347037

### References

<table>
<thead>
<tr>
<th>Journal</th>
<th>abID</th>
<th>Application</th>
<th>Species</th>
<th>PMID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature Communications (2019)</td>
<td>ab1</td>
<td>WB</td>
<td>Mouse</td>
<td>31391533</td>
</tr>
<tr>
<td>Cell (2019)</td>
<td>ab179483</td>
<td>WB</td>
<td>Mouse</td>
<td>31708126</td>
</tr>
<tr>
<td>Science Advances (2019)</td>
<td>ab2185</td>
<td>WB</td>
<td>Mouse</td>
<td>31281892</td>
</tr>
</tbody>
</table>