Western Blot Protocol Specific for HIF-2alpha Antibody (NB100-122)

General considerations for Western blot analysis of HIF-alpha proteins

1. HIF-2alpha is degraded under normoxic conditions and it is stabilized at O_2 concentrations below 5% or with treatment using certain agents (CoCl2, DFO, etc.).

2. Positive and negative controls should always be run side by side in a Western blot to accurately identify the protein band upregulated in the hypoxic sample.
3. (HepG2 Hypoxic ($CoCl_2$)/Normoxic Cell Lysate: NBP2-36451; HepG2 Hypoxic/Normoxic Cell Lysate: NBP2-36453).
4. To accurately compare treated and untreated samples and to ensure equal loading of samples the expression of a loading control should be evaluated.
   (alpha Tubulin Antibody (DM1A): NB100-690)

5. The fully post-translationally modified form of HIF-2alpha is ~118 kDa, or larger.

6. HIF-2alpha may form a heterodimer with HIF-1beta. However, this is not typically seeing under denaturing conditions.

Western Blot Protocol

Materials

1x Laemmli Sample Buffer: 2% SDS, 2.5% 2-mercaptoethanol (βME), 25% glycerol, 0.01% bromophenol blue, 62.5 mM Tris HC, pH 6.8

1X Running Buffer: 25 mM Tris-base, 192 mM glycine, 0.1% SDS. Adjust to pH 8.3

1X Transfer buffer (wet): 25 mM Tris-base, 192 mM glycine, 20% methanol.

1X TBS

TBST (1X TBS with 0.1% Tween-20)

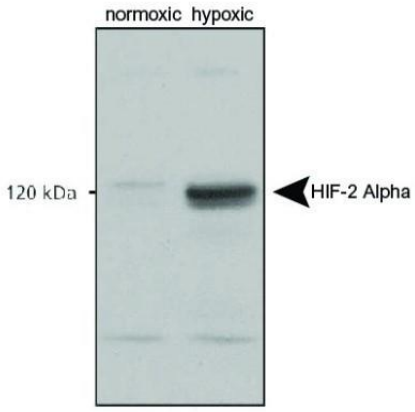
Blocking solution: TBST with 5% non-fat dry milk

Rabbit polyclonal anti-HIF-2 alpha primary antibody (NB100-122) in blocking solution (~1-2 μg/mL)

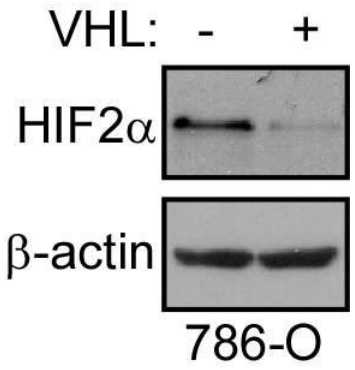
Methods

Whole-Cell Lysates

1. Load samples of treated and untreated cell lysates, 10-40 μg of total protein per lane on a 7.5% polyacrylamide gel (SDS-PAGE). Alternatively, gradient gels can be used for better resolution of lower molecular weight loading controls.
2. Resolve proteins by electrophoresis as required.
3. Transfer proteins to 0.45 μm PVDF membrane for 1 hour at 100V or equivalent.
4. Stain the blot using Ponceau S for 1-2 minutes to confirm efficient protein transfer onto the membrane.
5. Rinse the blot in distilled water to remove excess stain and mark the lanes and locations of molecular weight markers using a pencil.
6. Block the membrane using Blocking solution for 1 hour.
7. Dilute the rabbit anti-HIF-2 alpha primary antibody (NB100-122) in blocking solution (1-2 μg/ml) and incubate 1 hour at room temperature or overnight at 4°C.
8. Wash the membrane 3X 10 min in TBST.
9. Incubate in the appropriate diluted mouse-IgG HRP-conjugated secondary antibody in blocking solution (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the membrane 3X10 min in TBST.
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions (e.g., ECL, ECL Plus).
    Image blot.



Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Analysis using the HRP conjugate of NB100-122. Detection of normoxic and hypoxic nuclear rat cell lysates.



Genetic Strategies Validation. Western Blot: HIF-2 alpha/EPAS1 Antibody [NB100-122] - Western blot of 786-O cells without or with VHL overexpression. Image from verified customer review.