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NB100-105 Protocol

HIF-1 alpha Western Blot Info (NB100-105)

HIF-1 alpha Western Blot Info

1. The HIF proteins are among the most rapidly degrading proteins ever studied. Upon cellular re-oxygenation it can be completely degraded in less than 1 minute. Therefore, it is critical to prep only a few plates/dishes/flasks of cells at a time and to immediately place the cells into ice cold buffers and perform the whole protein prep on ice.
2. HIF-1 is largely undetectable in cells or tissues grown under normoxic conditions. It is stabilized only at O2 concentrations below 5% or with treatment using certain agents (CoCl2, DFO, etc.) so proper sample preparation is critical.
3. Upon stabilization HIF-1 translocates to the nucleus. The best western blots (cleanest) are always done using nuclear extracts. It is possible to detect HIF-1 in whole cell extracts, but they tend to be much dirtier and the staining is much weaker.
4. Finally, we recommend that a positive/negative control always be run side by side so that it is possible to discern which band is upregulated in the hypoxic sample. Unprocessed HIF1 is ~95 kDa while the fully post-translationally modified form is ~116 kDa, or larger. Additionally, HIF-1 alpha may form a heterodimer with HIF-1 beta (Duan, et al. Circulation. 2005;111:2227-2232.).

Depending on the sample, treatment, etc. you may see either a band or a doublet.
"EPO transcription can be activated by exposure of Hep3B cells to either hypoxia or cobalt chloride (7). HIF-1 binding activity was induced after 1 h and was maximal after 4-h treatment of Hep3B cells with 75 ,M cobalt chloride (Fig. 2A), which is similar to the kinetics of HIF-1 induction by hypoxia (data not shown). Exposure of HeLa cells to cobalt chloride for 4 h also induced HIF-1 activity. In contrast to hypoxia, which induced a doublet band corresponding to HIF-1 in EMSAs, cobalt chloride induced a single band of HIF-1 activity in both Hep3B and HeLa cells (compare Figs. 1A and 2A). We have not determined the basis for this reproducible difference in response to stimulation by hypoxia as compared to cobalt chloride" (Wang G, et al. (1993) PNAS 90, 4304-4308.).

Thus, it is critical to be able to look at upregulation compared to the control.