

## Product Information

### Monoclonal Anti-HIF-1 $\alpha$

#### Clone H1a67

Purified Mouse Immunoglobulin

Product Number **H 6536**

#### Product Description

Monoclonal Anti-HIF-1 $\alpha$  (Hypoxia Inducible Factor-1,  $\alpha$  subunit) (mouse IgG2b isotype) is derived from the hybridoma produced by the fusion of mouse myeloma NS1 cells with splenocytes from BALB/c mice immunized with amino acids 432-528 of the human HIF-1 $\alpha$  protein. The antibody is purified by Protein A/Protein G chromatography.

Monoclonal Anti-HIF-1 $\alpha$  recognizes the  $\alpha$  subunit (120 kDa) of human, sheep, mouse, rat, and ferret HIF-1. It has been used in immunoprecipitation, immunoblotting, and immunohistochemistry (frozen or formalin-fixed, paraffin-embedded tissue sections).

Hypoxia-inducible factor-1 (HIF-1) is a heterodimer composed of a 120 kDa HIF-1- $\alpha$  subunit complexed with a 91- to 94 kDa HIF-1- $\beta$  subunit. The predicted 826-amino acid HIF-1- $\alpha$  contains a bHLH (basic helix-loop-helix)-PAS domain at its N-terminus. Northern blot and Western blot analyses indicated that HIF-1 mRNAs and proteins are induced in cells exposed to 1% oxygen and decay rapidly upon return of the cells to 20% oxygen.<sup>1,2</sup>

HIF-1 is a MOP1 member of the PAS superfamily of transcription factors. It plays a pivotal role in cellular adaptation to changes in oxygen availability, including the regulation of genes involved in energy metabolism, angiogenesis, and apoptosis. HIF-1 activates transcription of hypoxia-inducible genes, including those encoding: erythropoietin, vascular endothelial growth factor (VEGF), heme oxygenase-1 inducible nitric oxide synthase, and the glycolytic enzymes aldolase A, enolase 1, lactate dehydrogenase A, phosphofructokinase I, and phosphoglycerate kinase 1.<sup>3</sup>

The  $\alpha$  subunits of HIF are rapidly degraded by the proteasome under normal conditions but are stabilized by hypoxia. Cobaltous ions or iron chelators mimic hypoxia, indicating that the stimuli may interact through effects on a ferroprotein oxygen sensor. In the presence of oxygen, HIF is targeted for destruction by an E3 ubiquitin ligase containing the von Hippel-Lindau tumor suppressor protein (pVHL). In VHL-defective cells, HIF- $\alpha$  subunits were constitutively stabilized and HIF-1 was activated. The interaction between HIF-1 and VHL is iron dependent and is necessary for the oxygen-dependent degradation of HIF- $\alpha$  subunits. These findings suggest that constitutive HIF-1 activation may underlie the angiogenic phenotype of VHL-associated tumors.<sup>4,5</sup>

Hypoxia-induced HIF-1- $\alpha$  activates expression of the gene encoding NIP3, which in turn primes cells for apoptosis under conditions of persistent oxygen deprivation. This pathway may play a role in cell death resulting from cerebral and myocardial ischemia. Recent studies identified a conserved HIF-VHL-prolyl hydroxylase pathway in *C. elegans* with Egl9 as a dioxygenase as main regulators of HIF by prolyl hydroxylation. In mammalian cells, they showed that three proteins, PHD1, PHD2, and PHD3, represent the HIF-prolyl hydroxylases with a conserved 2-histidine-1-carboxylate-iron coordination motif at the catalytic site. Direct modulation of recombinant enzyme activity by graded hypoxia, iron chelation, and cobaltous ions mirrored the characteristics of HIF induction *in vivo*, fulfilling requirements for these enzymes being oxygen sensors that regulate HIF.<sup>6,7</sup>

#### Reagent

Monoclonal Anti-HIF-1 $\alpha$  is supplied as a solution (~1 mg/mL) in phosphate buffered saline, pH 7.4, with 0.08% sodium azide as a preservative.

### Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

### Storage/Stability

Store at  $-20\text{ }^{\circ}\text{C}$ . Upon initial thawing, freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in a frost-free freezer. The antibody is stable for at least 12 months when stored appropriately. Working dilutions should be discarded if not used within 12 hours.

### Product Profile

By immunoblotting, the recommended working antibody concentration is 0.5-1.0  $\mu\text{g}/\text{mL}$ .

For immunoprecipitation, the recommended working antibody concentration is 2  $\mu\text{g}$  using 1 mg of protein lysate.

For immunohistochemistry, the recommended working antibody concentration is 2.0-4.0  $\mu\text{g}/\text{mL}$  using frozen or formalin-fixed, paraffin-embedded tissue sections.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining optimal working concentration by titration.

### References

1. Semenza, G.L., et al., Structural and functional analysis of hypoxia-inducible factor 1. *Kidney Int.*, **51**, 553-555 (1997).
2. Hogenesh, J.B., et al., Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. *J. Biol. Chem.*, **272**, 8581-8593 (1997).
3. Gassman, M., et al., Oxygen- and dioxin-regulation depends on heme-dependent oxygen sensing and assembly of interacting transcription factors. *Kidney Int.*, **51**, 567-574 (1997).
4. Maxwell, P.H., et al., The tumor suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*, **399**, 271-275 (1999).
5. Ivan, M., et al., HIF- $\alpha$  targeted for VHL-mediated destruction by proline hydroxylation: implications for  $\text{O}_2$  sensing. *Science*, **292**, 464-468 (2001).
6. Bruick, R.K., Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. *Proc. Nat. Acad. Sci. USA*, **97**, 9082-9087 (2000).
7. Epstein, A.C.R., et al., *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell*, **107**, 43-54 (2001).

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