Revision 3

Store at -20C

#3434

Hydroxy-HIF-1α (Pro564) (D43B5) XP® Rabbit mAb

Cell Signaling TECHNOLOGY®

Orders: 877-616-CELL (2355)
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Web: info@cellsignal.com
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3 Trask Lane | Danvers | Massachusetts | 01923 | USA

For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP, IF-IC	H Mk	Endogenous	120	Rabbit IgG	#Q16665	3091

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50
Immunofluorescence (Immunocytochemistry)	1:3200 - 1:6400

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity/Sensitivity

Hydroxy-HIF-1α (Pro564) (D43B5) XP® Rabbit mAb detects endogenous levels of HIF-1α only when hydroxylated at Pro564. This antibody may cross react with other overexpressed proline hydroxylated proteins.

Species predicted to react based on 100% sequence homology

Mouse, Rat, Chicken, Xenopus, Zebrafish, Pig

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic hydroxypeptide corresponding to residues surrounding Pro564 of human HIF-1α.

Background

Hypoxia-inducible factor 1 (HIF1) is a heterodimeric transcription factor that plays a critical role in the cellular response to hypoxia (1). The HIF1 complex consists of two subunits, HIF-1α and HIF-1β, which are basic helix-loop-helix proteins of the PAS (Per, ARNT, Sim) family (2). HIF1 regulates the transcription of a broad range of genes that facilitate responses to the hypoxic environment, including genes regulating angiogenesis, erythropoiesis, cell cycle, metabolism, and apoptosis. The widely expressed HIF-1α is typically degraded rapidly in normoxic cells by the ubiquitin/proteasomal pathway. Under normoxic conditions, HIF-1α is proline hydroxylated leading to a conformational change that promotes binding to the von Hippel-Lindau protein (VHL) E3 ligase complex; ubiquitination and proteasomal degradation follows (3,4). Both hypoxic conditions and chemical hydroxylase inhibitors (such as desferrioxamine and cobalt) inhibit HIF-1α degradation and lead to its stabilization. In addition, HIF-1α can be induced in an oxygen-independent manner by various cytokines through the PI3K-AKT-mTOR pathway (5-7).

HIF-1β is also known as AhR nuclear translocator (ARNT) due to its ability to partner with the aryl hydrocarbon receptor (AhR) to form a heterodimeric transcription factor complex (8). Together with AhR, HIF-1β plays an important role in xenobiotics metabolism (8). In addition, a chromosomal translocation leading to a TEL-ARNT fusion protein is associated with acute myeloblastic leukemia (9). Studies also found that ARNT/HIF-1β expression levels decrease significantly in pancreatic islets from patients with type 2 diabetes, suggesting that HIF-1β plays an important role in pancreatic β-cell function (10).~Two critical prolines in HIF-1α (Pro564 and Pro402) can be hydroxylated by proline hydroxylase under normoxia conditions. Hydroxylation of HIF-1α leads to its binding to VHL and ubiquitin mediated degradation (3,11,12).

Background References

1. Sharp, F.R. and Bernaudin, M. (2004) *Nat Rev Neurosci* 5, 437-48.
2. Wang, G.L. et al. (1995) *Proc Natl Acad Sci U S A* 92, 5510-4.
3. Jaakkola, P. et al. (2001) *Science* 292, 468-72.
4. Maxwell, P.H. et al. (1999) *Nature* 399, 271-5.
5. Fukuda, R. et al. (2002) *J Biol Chem* 277, 38205-11.
6. Jiang, B.H. et al. (2001) *Cell Growth Differ* 12, 363-9.
7. Laughner, E. et al. (2001) *Mol Cell Biol* 21, 3995-4004.
8. Walisser, J.A. et al. (2004) *Proc Natl Acad Sci U S A* 101, 16677-82.
9. Salomon-Nguyen, F. et al. (2000) *Proc Natl Acad Sci U S A* 97, 6757-62.
10. Gunton, J.E. et al. (2005) *Cell* 122, 337-49.
11. Ivan, M. et al. (2001) *Science* 292, 464-8.
12. Masson, N. et al. (2001) *EMBO J* 20, 5197-206.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **IP:** Immunoprecipitation **IF-IC:** Immunofluorescence (Immunocytochemistry)

Cross-Reactivity Key

H: Human **Mk:** Monkey

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Limited Uses