Store at -20°C

Hypoxia Pathway Antibody Sampler Kit

#15792

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
HIF-1α (D1S7W) XP® Rabbit mAb	36169	20 µl	120 kDa	Rabbit IgG
Hydroxy-HIF-1α (Pro564) (D43B5) XP® Rabbit mAb	3434	20 µl	120 kDa	Rabbit IgG
HIF-1β/ARNT (D28F3) XP® Rabbit mAb	5537	20 µl	87 kDa	Rabbit IgG
HIF-2α (D6T8V) Rabbit mAb	59973	20 µl	120 kDa	Rabbit IgG
FIH (D19B3) Rabbit mAb	4426	20 µl	42 kDa	Rabbit IgG
PHD-2/Egln1 (D31E11) Rabbit mAb	4835	20 µl	50 kDa	Rabbit IgG
VHL Antibody	68547	20 µl	18-22 kDa	Rabbit
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

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 antibodies.*

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

Description: The Hypoxia Pathway Antibody Sampler Kit provides an economical means to investigate select proteins involved in the hypoxia pathway. The kit contains enough primary antibodies to perform two western blot experiments with each primary antibody.

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).

Hypoxia-inducible factor (HIF) is essential for the cellular response to hypoxia (11,12). There are several isoforms of the HIF α subunit. Studies have found that HIF-1α and HIF-2α expression is increased in some human cancers. HIF-1α has both pro- and anti-proliferative activities, whereas HIF-2α does not possess anti-proliferative activity (12). Therefore, HIF-2α likely plays an important role in tumorigenesis (12,13).

FIH (Factor inhibiting HIF-1, HIF asparagine hydroxylase) is a dioxygen-dependent asparaginyl hydroxylase that modifies target protein function by hydroxylating target protein asparagine residues (14-16). HIF, a transcriptional activator involved in control of cell cycle in response to hypoxic conditions, is an important target for FIH regulation. FIH functions as an oxygen sensor that regulates HIF function by hydroxylating at Asn803 in the carboxy-terminal transactivation domain (CAD) of HIF (17,18). During normoxia, FIH uses cellular oxygen to hydroxylate HIF-1 and prevent interaction of HIF-1 with transcriptional coactivators, including the CBP/p300-interacting transactivator. Under hypoxic conditions, FIH remains inactive and does not inhibit HIF, allowing the activator to regulate transcription of genes in response to low oxygen conditions (17-19). FIH activity is regulated through interaction with proteins, including Siah-1, which targets FIH for proteasomal degradation (20). The Cut-like homeodomain protein CDP can bind the FIH promoter region to regulate FIH expression at the transcriptional level (21). Phosphorylation of HIF at Thr796 also can prevent FIH hydroxylation on Asn803 (22). Potential FIH substrates also include proteins with ankyrin repeat domains, such as Iκ-B, Notch, and ASB4 (23-25).

PHD1 (Egln2), PHD-2 (Egln1), and PHD3 (Egln3) are members of the Egln family of proline hydroxylases. They function as oxygen sensors that catalyze the hydroxylation of HIF on prolines 564 and 402, initiating the first step of HIF degradation through the VHL/ubiquitin pathway (26,27).

Specificity/Sensitivity: HIF-1α (D1S7W) XP® Rabbit mAb recognizes endogenous levels of total HIF-1α protein. This antibody does not cross-react with HIF-2α protein. 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. HIF-1β/ARNT (D28F3) XP® Rabbit mAb detects endogenous levels of total HIF-1β/ARNT protein. HIF-2α (D6T8V) Rabbit mAb recognizes endogenous levels of total HIF-2α protein. This antibody does not cross-react with HIF-1α protein. FIH (D19B3) Rabbit mAb detects endogenous levels of total FIH protein. PHD-2/Egln1 (D31E11) Rabbit mAb detects endogenous levels of total PHD-2/Egln1 protein. VHL Antibody recognizes endogenous levels of total VHL protein.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Leu478 of human HIF-1α protein, Gly688 of human HIF-2α protein, Tyr35 of human FIH protein, and Val226 of human PHD-2/Egln1 protein. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the sequence around Ile479 of human HIF-1β/ARNT protein. Monoclonal antibody is produced by immunizing animals with a synthetic hydroxypeptide corresponding to residues surrounding Pro564 of human HIF-1α. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy terminus of human VHL protein.

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Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity: H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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