Revision 1

Store at -20C

#43065

Hypoxia Activation IHC Antibody Sampler Kit

1 Kit (6 x 20 microliters)

Cell Signaling TECHNOLOGY®

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Product Includes	Product #	Quantity	Mol. Wt	Isotype/Source
HIF-1α (E1V6A) Rabbit mAb	48085	20 µl	120 kDa	Rabbit IgG
HIF-1β/ARNT (D28F3) XP® Rabbit mAb	5537	20 µl	87 kDa	Rabbit IgG
VHL (E3X9K) Rabbit mAb	81292	20 µl		Rabbit IgG
p300 (D8Z4E) Rabbit mAb	86377	20 µl	300 kDa	Rabbit IgG
SirT1 (1F3) Mouse mAb	8469	20 µl	120 kDa	Mouse IgG1
GSK-3β (D5C5Z) XP® Rabbit mAb	12456	20 µl	46 kDa	Rabbit IgG
PKM2 (D78A4) XP® Rabbit mAb	4053	20 µl	60 kDa	Rabbit IgG
LDHA (C4B5) Rabbit mAb	3582	20 µl	37 kDa	Rabbit IgG
Glut1 (E4S6I) Rabbit mAb	73015	20 µl	45-60 kDa	Rabbit IgG

Please visit cellsignal.com for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

Description

The Hypoxia Activation IHC Antibody Sampler Kit provides an economical means of detecting select components involved in the regulation of HIF-1α, select components regulated by HIF-1α, and HIF-1β/ARNT protein in formalin-fixed, paraffin-embedded tissue samples.

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.*

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). CBP (CREB-binding protein) and p300 are highly conserved and functionally related transcriptional co-activators that associate with transcriptional regulators and signaling molecules, integrating multiple signal transduction pathways with the transcriptional machinery (11,12). CBP/p300 also contain histone acetyltransferase (HAT) activity, allowing them to acetylate histones and other proteins (12). The Silent Information Regulator (SIR2) family of genes is a highly conserved group of genes that encode nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylases, also known as class III histone deacetylases. The first discovered and best characterized of these genes is *Saccharomyces cerevisiae SIR2*, which is involved in silencing of mating type loci, telomere maintenance, DNA damage response, and cell aging (13). SirT1, the mammalian ortholog of Sir2, is a nuclear protein implicated in the regulation of many cellular processes, including apoptosis, cellular senescence, endocrine signaling, glucose homeostasis, aging, and longevity. Targets of SirT1 include acetylated p53 (14,15), p300 (16), Ku70 (17), forkhead (FoxO) transcription factors (17,18), PPARγ (19), and the PPARγ coactivator-1α (PGC-1α) protein (20). Glycogen synthase kinase-3 (GSK-3) was initially identified as an enzyme that regulates glycogen synthesis in response to insulin (21). GSK-3 is a ubiquitously expressed serine/threonine protein kinase that phosphorylates and inactivates glycogen synthase. GSK-3 is a critical downstream element of the PI3K/Akt cell survival pathway whose activity can be inhibited by Akt-mediated phosphorylation at Ser21 of GSK-3α and Ser9 of GSK-3β (22,23). Pyruvate kinase is a glycolytic enzyme that catalyzes the conversion of phosphoenolpyruvate to

pyruvate. In mammals, the M2 isoform (PKM2) is expressed during embryonic development (24). Lactate dehydrogenase (LDH) catalyzes the interconversion of pyruvate and NADH to lactate and NAD^+. The major form of LDH found in muscle cells is the A (LDHA) isozyme (25). Glucose transporter 1 (Glut1, SLC2A1) is a widely expressed transport protein that transports a number of different aldose sugars into cells (26,27).

Background References

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