Revision 1

Store at -20C

#42022

Insulin/IGF-1 Signaling Pathway Antibody Sampler Kit

1 Kit (9 x 20 microliters)

Cell Signaling TECHNOLOGY®

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Product Includes	Product #	Quantity	Mol. Wt	Isotype/Source
Insulin Receptor β (4B8) Rabbit mAb	3025	20 µl	95 kDa	Rabbit IgG
IGF-I Receptor β (D23H3) XP® Rabbit mAb	9750	20 µl	95 kDa	Rabbit IgG
Phospho-IGF-I Receptor β (Tyr1131)/Insulin Receptor β (Tyr1146) Antibody	3021	20 µl	95 kDa	Rabbit
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	4060	20 µl	60 kDa	Rabbit IgG
Phospho-Akt (Thr308) (D25E6) XP® Rabbit mAb	13038	20 µl	60 kDa	Rabbit IgG
Phospho-GSK-3β (Ser9) (D85E12) XP® Rabbit mAb	5558	20 µl	46 kDa	Rabbit IgG
Phospho-FoxO1 (Thr24)/FoxO3a (Thr32)/FoxO4 (Thr28) (4G6) Rabbit mAb	2599	20 µl	65, 78 to 82, 95 kDa	Rabbit
Phospho-mTOR (Ser2448) (D9C2) XP® Rabbit mAb	5536	20 µl	289 kDa	Rabbit IgG
Phospho-Tuberin/TSC2 (Ser939) Antibody	3615	20 µl	200 kDa	Rabbit
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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

Description

The Insulin/IGF-1 Signaling Pathway Antibody Sampler Kit provides an economical means of detecting select components involved in the insulin and/or IGF-1 signaling pathways. The kit contains enough primary antibodies to perform at least two western blot experiments per antibody.

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.

Background

Insulin and IGF-1 act on two closely related tyrosine kinase receptors to initiate a cascade of signaling events. These signaling events activate a variety of biological molecules, including kinases and transcription factors, which regulate cell growth, survival and metabolism.

Type I insulin-like growth factor receptor (IGF-IR) is a transmembrane receptor tyrosine kinase that is widely expressed in many cell lines and cell types within fetal and postnatal tissues (1-3). Three tyrosine residues within the kinase domain (Tyr1131, Tyr1135, and Tyr1136) are the earliest major autophosphorylation sites (4). Phosphorylation of these three tyrosine residues is necessary for kinase activation (5,6). Insulin receptors (IRs) share significant structural and functional similarity with IGF-I receptors, including the presence of an equivalent tyrosine cluster (Tyr1146/1150/1151) within the kinase domain activation loop. Tyrosine autophosphorylation of IRs is one of the earliest cellular responses to insulin stimulation (7). Autophosphorylation begins with phosphorylation at Tyr1146 and either Tyr1150 or Tyr1151, while full kinase activation requires triple tyrosine phosphorylation (8).

Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (9-11). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (10,11). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (12) and by phosphorylation within the carboxy terminus at Ser473. The previously elusive PDK2 responsible for phosphorylation of Akt at Ser473 has been identified as mammalian target of rapamycin (mTOR) in a rapamycin-insensitive complex with rictor and Sin1 (13,14).

Tuberin is a product of the TSC2 tumor suppressor gene and an important regulator of cell proliferation and tumor development (15). Tuberin is phosphorylated on Ser939 and Thr1462 in response to PI3K activation and the human TSC complex is a direct biochemical target of the PI3K/Akt pathway (16). This result complements Drosophila genetics studies suggesting the possible involvement of the tuberin-hamartin complex in the PI3K/Akt mediated insulin pathway (17-19).

The mammalian target of rapamycin (mTOR, FRAP, RAFT) is a Ser/Thr protein kinase (20-22) that functions as an ATP and amino acid sensor to balance nutrient availability and cell growth (23,24). When sufficient nutrients are available, mTOR responds to a phosphatidic acid-mediated signal to

transmit a positive signal to p70 S6 kinase and participate in the inactivation of the eIF4E inhibitor, 4E-BP1 (25). These events result in the translation of specific mRNA subpopulations. mTOR is phosphorylated at Ser2448 via the PI3 kinase/Akt signaling pathway and autophosphorylated at Ser2481 (26,27).

The Forkhead family of transcription factors is involved in tumorigenesis of rhabdomyosarcoma and acute leukemias (28-30). Within the family, three members (FoxO1, FoxO4, and FoxO3a) have sequence similarity to the nematode orthologue DAF-16, which mediates signaling via a pathway involving IGFR1, PI3K, and Akt (31-33). Active forkhead members act as tumor suppressors by promoting cell cycle arrest and apoptosis. Increased proliferation results when forkhead transcription factors are inactivated through phosphorylation by Akt at Thr24, Ser256, and Ser319, which results in nuclear export and inhibition of transcription factor activity (34).

Glycogen synthase kinase-3 (GSK-3) was initially identified as an enzyme that regulates glycogen synthesis in response to insulin (35). 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 (36,37).

Background References

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