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#42022

# Insulin/IGF-1 Signaling Pathway Antibody Sampler Kit

1 Kit  $\mu$ l  
(9 x 20  $\mu$ l)



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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Insulin Receptor beta(4B8) Rabbit mAb	3025	20 $\mu$ l	95 kDa	Rabbit IgG
IGF-IR beta (D23H3) XP <sup>®</sup> Rabbit mAb	9750	20 $\mu$ l	95 kDa	Rabbit IgG
P-IGF-IR beta(Y1131)/InsR beta (Y1146) Rabbit Ab	3021	20 $\mu$ l	95 kDa	Rabbit
P-Akt (S473) (D9E) XP <sup>®</sup> Rabbit mAb	4060	20 $\mu$ l	60 kDa	Rabbit IgG
P-Akt (T308) (D25E6) XP <sup>®</sup> Rabbit mAb	13038	20 $\mu$ l	60 kDa	Rabbit IgG
P-GSK-3-beta (S9) (D85E12) XP <sup>®</sup> Rabbit mAb	5558	20 $\mu$ l	46 kDa	Rabbit IgG
P-FoxO1 (T24)/FoxO3a (T32)/FoxO4 (T28) (4G6) Rabbit mAb	2599	20 $\mu$ l	65, 78 to 82, 95 kDa	Rabbit
Phospho-mTOR (Ser2448) (D9C2) XP <sup>®</sup> Rabbit mAb	5536	20 $\mu$ l	289 kDa	Rabbit IgG
P-Tuberin/TSC2 (S939) Rabbit Ab	3615	20 $\mu$ l	200 kDa	Rabbit
Anti-Rabbit IgG, HRP-linked Antibody	7074	100 $\mu$ l		Goat

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

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

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibodies.

U.S. Patent No. 7,429,487, foreign equivalents, and child patents deriving therefrom.

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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.**

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 IGF1R, 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-3 $\alpha$  and Ser9 of GSK-3 $\beta$  (36,37).

**Specificity/Sensitivity:** Insulin Receptor beta (4B8) Rabbit mAb detects endogenous levels of total insulin receptor  $\beta$ . It does not cross-react with IGF-IR  $\beta$ . IGF-I Receptor  $\beta$  (D23H3) XP<sup>®</sup> Rabbit mAb detects endogenous levels of total IGF-I receptor  $\beta$  protein. This antibody does not cross-react with insulin receptor. Phospho-IGF-I Receptor  $\beta$  (Tyr1131)/Insulin Receptor  $\beta$  (Tyr1146) Antibody detects endogenous levels of Tyr1131-phosphorylated IGF-I receptor and Tyr1146-phosphorylated insulin receptor. The antibody cross-reacts with activated PDGF, FGF and EGF receptors, ErbB2 and c-Met. Phospho-Akt (Ser473) (D9E) XP<sup>®</sup> Rabbit mAb detects endogenous levels of Akt only when phosphorylated at Ser473. Phospho-Akt (Thr308) (D25E6) XP<sup>®</sup> Rabbit mAb recognizes endogenous levels of Akt1 protein only when phosphorylated at Thr308. This antibody also recognizes endogenous levels of Akt2 protein when phosphorylated at Thr309 or Akt3 protein when phosphorylated at Thr305. Phospho-GSK-3 $\beta$  (Ser9) (D85E12) XP<sup>®</sup> Rabbit mAb detects endogenous levels of GSK-3 $\beta$  only when phosphorylated at Ser9. This antibody reacts with denatured components of bovine serum, including BSA. Phospho-Tuberin/TSC2 (Ser939) Antibody detects endogenous levels of tuberlin only when phosphorylated at serine 939. This antibody does not cross-react with tuberlin phosphorylated at other sites. Phospho-FoxO1 (Thr24)/FoxO3a (Thr32)/FoxO4 (Thr28) (4G6) Rabbit mAb detects endogenous levels of FoxO1 when phosphorylated at Thr24, of FoxO3a when phosphorylated at Thr32 or FoxO4 when phosphorylated at Thr28. Phospho-mTOR (Ser2448) (D9C2) XP<sup>®</sup> Rabbit mAb detects endogenous levels of mTOR protein only when phosphorylated at Ser2448.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Tyr999 of human insulin receptor  $\beta$ . Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy terminus of human IGF-I receptor  $\beta$  protein. Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues of human IGF-I Receptor  $\beta$ . Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Ser473 of human Akt. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Thr308 of human Akt1 protein. Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser9 of human GSK-3 $\beta$ . Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Ser939 of human tuberlin. Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr28 of human FoxO4. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser2448 of human mTOR protein.

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