Protein Synthesis Phosphorylation Sampler Kit

E051012

Kits Includes	Cat.	Quantity	Application	Reactivity	Source
Akt (Phospho-Ser473) Antibody	E011054-1	50μg/50μl	IHC, WB	Human, Mouse, Rat	Rabbit
AMPK1/AMPK2 (Phospho-Ser485/Ser491) Antibody	E011174-1	50μg/50μl	IHC, WB	Human, Mouse, Rat	Rabbit
mTOR(Phospho-Ser2448) Antibody	E011221-1	50μg/50μl	IHC	Human, Mouse, Rat	Rabbit
p70 S6 Kinase (Phospho-Thr421) Antibody	E011254-1	50μg/50μl	IHC, WB	Human, Mouse, Rat	Rabbit
PDK1 (Phospho-Ser241) Antibody	E011005-1	50μg/50μl	IHC, WB,IF	Human, Mouse, Rat	Rabbit

The serine-threonine protein kinase encoded by the **AKT**1 gene is catalytically inactive in serum-starved primary and immortalized fibroblasts. **AKT**1 and the related **AKT**2 are activated by platelet-derived growth factor. The activation is rapid and specific, and it is abrogated by mutations in the pleckstrin homology domain of **AKT**1. It was shown that the activation occurs through phosphatidylinositol 3-kinase. In the developing nervous system **AKT** is a critical mediator of growth factor-induced neuronal survival. Survival factors can suppress apoptosis in a transcription-independent manner by activating the serine/threonine kinase **AKT**1, which then phosphorylates and inactivates components of the apoptotic machinery. Multiple alternatively spliced transcript variants have been found for this gene. General protein kinase capable of phosphorylating several known proteins. Phosphorylates TBC1D4. Signals downstream of phosphatidylinositol 3-kinase (PI(3)K) to mediate the effects of various growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin and insulin-like growth factor I (IGF-I). Plays a role in glucose transport by mediating insulin-induced translocation of the GLUT4 glucose transporter to the cell surface. Mediates the antiapoptotic effects of IGF-I. Mediates insulin-stimulated protein synthesis by phosphorylating TSC2 at 'Ser-939' and 'Thr-1462', thereby activating mTORC1 signaling and leading to both phosphorylation of 4E-BP1 and in activation of RPS6KB1. Promotes glycogen synthesis by mediating the insulin-induced activation of glycogen synthase

Akt (Protein kinase B, PKB) is a serine/threonine kinase that plays a key in regulating cell survival, insulin signaling, angiogenesis and tumor formation. Akt is a downstream mediator of the PI 3-K pathway, resulting in the recruitment of Akt to the plasma membrane via the PH (plexstrin homology domain) of Akt. Akt is fully activated by phosphorylation at two key sites: Ser308 (phosphorylated by PDK1) and Thr478 (phosphorylated by mTOR and DNA-PK). Akt can then phosphorylated a wide range of substrates including transcription factors (e.g. FOXO1), kinases (GSK-3, Raf-1, ASK, Chk1) and other proteins with important signaling roles (e.g. Bad, MDM2). There are three

isoforms of Akt; Akt 1, 2 and 3 (also known as PKBalpha, beta and gamma). Phosphorylation of AKT on Thr-308, Ser-473 and Tyr-474 is required for full activity. Ser-473 phosphorylation by mTORC2 favors, Thr-308 phosphorylation by PDPK1. Ser-473 phosphorylation is enhanced by interaction with AGAP2 isoform 2 (PIKE-A). Ser-473 phosphorylation is enhanced in focal cortical dysplasias with Taylor-type balloon cells.

The protein encoded by this gene belongs to the ser/thr protein kinase family. It is the catalytic subunit of the 5'-prime-AMP-activated protein kinase (**AMPK**). **AMPK** is a cellular energy sensor conserved in all eukaryotic cells. The kinase activity of **AMPK** is activated by the stimuli that increase the cellular AMP/ATP ratio. **AMPK** regulates the activities of a number of key metabolic enzymes through phosphorylation. It protects cells from stresses that cause ATP depletion by switching off ATP-consuming biosynthetic pathways. Alternatively spliced transcript variants encoding distinct isoforms have been observed. Responsible for the regulation of fatty acid synthesis by phosphorylation of acetyl-CoA carboxylase. It also regulates cholesterol synthesis via phosphorylation and inactivation of hormone-sensitive lipase and hydroxymethylglutaryl-CoA reductase. Appears to act as a metabolic stress-sensing protein kinase switching off biosynthetic pathways when cellular ATP levels are depleted and when 5'-AMP rises in response to fuel limitation and/or hypoxia. This is a catalytic subunit.

AMP-activated protein kinase (AMPK) is a heterodimeric protein serine/threonine kinase that is composed of alpha-(catalytic) and beta/gamma- (regulatory) subunits. AMPK acts as a sensor of the energy status of cells and ensures survival at times of metabolic stress. AMPK phosphorylates many metabolic enzymes to stimulate catabolic pathways, such as ketogenesis, and inhibit anabolic pathways, such as protein synthesis. The long-term activation of AMPK increases the capacity of cells to produce ATP. AMPK is regulated by phosphorylation at the Thr-172 residue of the alpha-subunit by AMPKK and by phosphorylation by calmodulin-dependent protein kinase kinase-beta (CamKKbeta). In addition, the ratio of AMP:ATP mediates allosteric activation of the enzyme. AMPK is found throughout the body with high concentrations in metabolically active tissues such as the skeletal muscles and liver. The protein encoded by this gene belongs to a family of phosphatidylinositol kinase-related kinases. These kinases mediate cellular responses to stresses such as DNA damage and nutrient deprivation. This protein acts as the target for the cell-cycle arrest and immunosuppressive effects of the FKBP12-rapamycin complex. The ANGPTL7 gene is located in an intron of this gene. Kinase subunit of both **mTOR**C1 and **mTOR**C2, which regulate cell growth and survival in response to nutrient and hormonal signals. **mTOR**C1 is activated in response to growth factors or amino-acids. Amino-acid-signaling to **mTOR**C1 is mediated by Rag GTPases, which cause amino-acid-induced relocalization of **mTOR** within the endomembrane system. Growth factor-stimulated **mTOR**C1 activation involves AKT1-mediated phosphorylation of TSC1-TSC2, which leads to the activation of the RHEB GTPase that potently activates the protein kinase activity of **mTOR**C1. Activated **mTOR**C1 up-regulates protein synthesis by phosphorylating key regulators of mRNA translation and ribosome synthesis. **mTOR**C1

phosphorylates EIF4EBP1 and releases it from inhibiting the elongation initiation factor 4E (eiF4E). **mTOR**C1 phosphorylates and activates S6K1 at 'Thr-421', which then promotes protein synthesis by phosphorylating PDCD4 and targeting it for degradation. **mTOR**C2 is also activated by growth factors, but seems to be nutrient-insensitive. **mTOR**C2 seems to function upstream of Rho GTPases to regulate the actin cytoskeleton, probably by activating one or more Rho-type guanine nucleotide exchange factors. **mTOR**C2 promotes the serum-induced formation of stress-fibers or F-actin. **mTOR**C2 plays a critical role in AKT1 'Ser-473' phosphorylation, which may facilitate the phosphorylation of the activation loop of AKT1 on 'Thr-308' by PDK1 which is a prerequisite for full activation. **mTOR**C2 regulates the phosphorylation of SGK1 at 'Ser-422'. **mTOR**C2 also modulates the phosphorylation of PRKCA on 'Ser-657'.

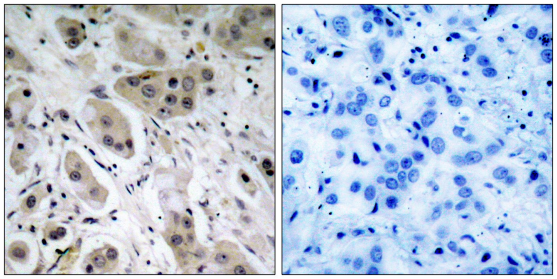
RPS6KB1 gene encodes a member of the RSK (ribosomal S6 kinase) family of serine/threonine kinases. This kinase contains 2 non-identical kinase catalytic domains and phosphorylates several residues of the S6 ribosomal protein. The kinase activity of this protein leads to an increase in protein synthesis and cell proliferation. Amplification of the region of DNA encoding this gene and overexpression of this kinase are seen in some breast cancer cell lines. Alternate translational start sites have been described and alternate transcriptional splice variants have been observed but have not been thoroughly characterized. Phosphorylates specifically ribosomal protein S6 in response to insulin or several classes of mitogens.Promotes protein synthesis by phosphorylating PDCD4 at 'Ser-67' and targeting it for degradation

Pyruvate dehydrogenase (PDH) is a mitochondrial multienzyme complex that catalyzes the oxidative decarboxylation of pyruvate and is one of the major enzymes responsible for the regulation of homeostasis of carbohydrate fuels in mammals. The enzymatic activity is regulated by a phosphorylation/dephosphorylation cycle. Phosphorylation of PDH by a specific pyruvate dehydrogenase kinase (PDK) results in inactivation. Inhibits the mitochondrial pyruvate dehydrogenase complex by phosphorylation of the E1 alpha subunit, thus contributing to the regulation of glucose metabolism.

Akt (Phospho-Ser473) Antibody

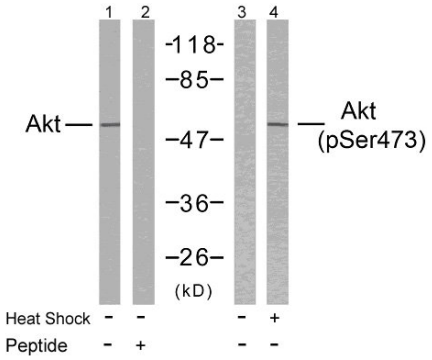
E011054

Catalog Number: E011054-1, E011054-2
Amount: 50μg/50μl, 100μg/100μl
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human Akt around the phosphorylation site of serine 473 (Q-F-S^P-Y-S).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatogramphy using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: Akt (phospho-Ser473) antibody detects endogenous levels of Akt only when phosphorylated at serine 473.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100
Swiss-Prot No. : P31749
References: Baudhuin LM, et al. (2004) FASEB J Feb; 18(2): 341-3.
Min YH, et al. (2004) Cancer Res; 64(15): 5225-31.
Feng J, et al. (2004) J Biol Chem; 279(34): 35510-7.



P-Peptide - +

Immunohistochemical analysis of paraffin- embedded human breast carcinoma tissue, using Akt (phospho-Ser473) antibody (E011054).

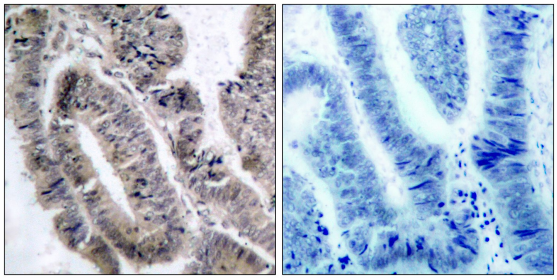


Western blot analysis of extract from HeLa cells untreated or treated with heat shock using Akt (Ab-473) antibody (E021054, Lane 1 and 2) and Akt (phospho-Ser473) antibody (E011054, Lane 3 and 4).

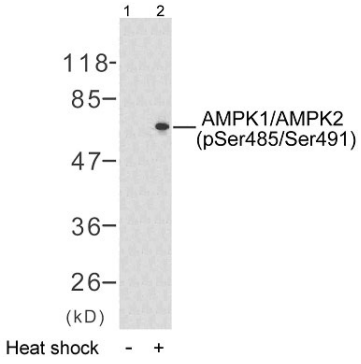
AMPK1/AMPK2 (Phospho-Ser485/Ser491)

E011174

Catalog Number: E011174-1, E011174-2
Amount: 50μg/50μl, 100μg/100μl
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human AMPK1/AMPK2 around the phosphorylation site of serine 485/491 (S-G- S^{P}-V-S).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatography using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: AMPK1/AMPK2 (phospho-Ser485/Ser491) antibody detects endogenous levels of AMPK1/AMPK2 only when phosphorylated at serine 485 or serine 491.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100
Swiss-Prot No. : Q13131 P54646
References: Kim JE, et al. (2005) J Proteome Res. 4(4): 1339-1346.
Woods A, et al. (2003) J Biol Chem. 278(31): 28434-28442.



Immunohistochemical analysis of paraffin- embedded human colon carcinoma tissue, using AMPK1/AMPK2 (phospho-Ser485/Ser491) antibody (E011174).

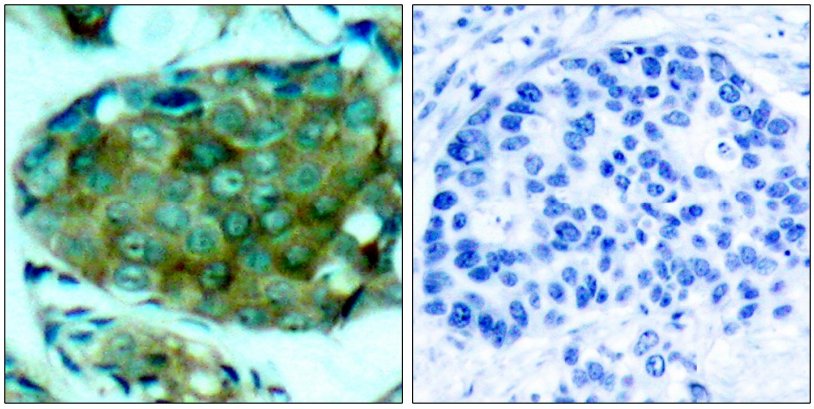


Western blot analysis of extract from HeLa cells untreated or treated with heat shock (30min), using AMPK1/AMPK2 (phospho-Ser485/Ser491) antibody (E011174).

mTOR (Phospho-Ser2448) Antibody

E011221

Catalog Number: E011221-1, E011221-2
Amount: 50μg/50μl, 100μg/100μl
Swiss-Prot No. : P42345
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human mTOR around the phosphorylation site of serine 2448 (T-D-S^{P}-Y-S).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatogramphy using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: mTOR (phospho-Ser2448) antibody detects endogenous levels of mTOR only when phosphorylated at serine 2448.
Reactivity: Human, Mouse, Rat
Applications: IHC: 1:50~1:100
References:
Holz MK, et,al. (2005) J Biol Chem ;280:26089-26093
Chiang GG, et,al. (2005) J Biol Chem ;280: 25485-25490
Mothe-Satney I, et,al. (2004) J Biol Chem ;279: 42628-42637
Bolster DR, et,al. (2003) J Physiol ;553:213-220.



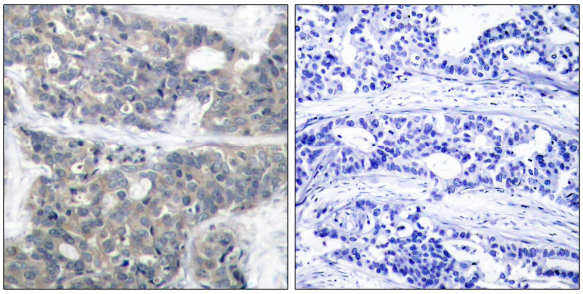
P-Peptide - +

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue, using mTOR (phospho-Ser2448) antibody (E011221).

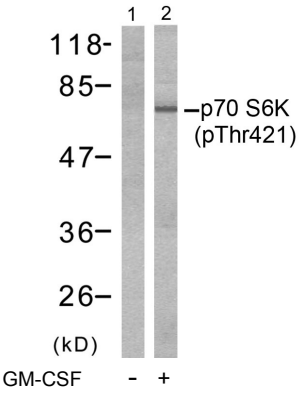
p70 S6 Kinase (Phospho-Thr421) Antibody

E011254

Catalog Number: E011254-1, E011254-2
Amount: 50μg/50μl, 100μg/100μl
Swiss-Prot No. : P23443
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human p70 S6 Kinase around the phosphorylation site of threonine 421 (P-R-T^P-P-V).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatogramphy using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: p70 S6 Kinase (phospho-Ser421) antibody detects endogenous levels of p70 S6 Kinase only when phosphorylated at threonine 421.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50-1:100
References: Xiao-Feng, et al. (2003) Le1 30 Volume 22: 484-497
An WL, et al. (2003) Am J Pathol. 163(2): 591-607.
Le XF, et al. (2003) Oncogene.22(4): 484-97



Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using p70 S6 Kinase (phospho-Thr421) antibody (E011254).

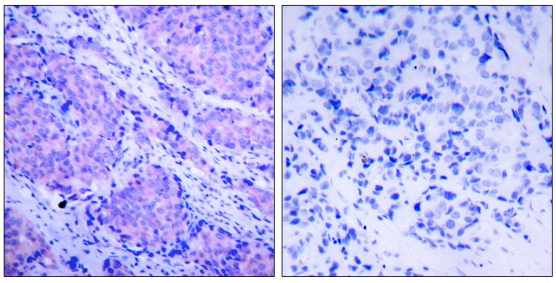


Western blot analysis of extracts from Jurkat cells, untreated or treated with GM-CSF (25ng/ml 30min) using p70 S6 Kinase (phospho-Thr421) antibody (E011254, Line 1 and 2).

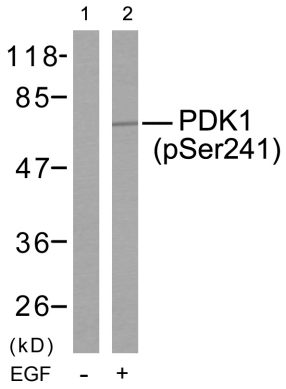
PDK1 (Phospho-Ser241) Antibody

E011005

Catalog Number: E011005-1, E011005-2
Amount: 50μg/50μl, 100μg/100μl
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human PDK1 around the phosphorylation site of serine 241 (A-N-S^P-F-V).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatogramphy using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: PDK1 (phospho-Ser241) antibody detects endogenous levels of PDK1 only when phosphorylated at serine 241.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100 IF: 1:100-200
Swiss-Prot No. : O15530
References:
Scheid MP,et al. (2005)Mol Cell Biol; 25(6): 2347-63
Chen H, et al. (2001) Biochemistry; 40(39): 11851-9
Sato S,et al. (2002) J Biol Chem; 277(42): 39360-7
Lim MA, et al.(2003)Proc Natl Acad Sci U S A; 100(24): 14006-11



Immunohistochemical analysis of paraffin- embedded human breast carcinoma tissue using PDK1 (phospho-Ser241) antibody (E011005).



Western blot analysis of extracts from MDA-MB-435 cells using PDK1 (phospho-Ser241) antibody (E011005).