Phospho-β-Catenin Pathway Sampler Kit

E051024

Kits Includes	Cat.	Quantity	Application	Reactivity	Source
Akt (Phospho-Ser473) Antibody	E011054-1	50μg/50μl	IHC, WB	Human, Mouse, Rat	Rabbit
β -Catenin (Phospho-Ser37) Antibody	E011219-1	50μg/50μl	IHC, WB	Human, Mouse, Rat	Rabbit
GSK3α (Phospho-Ser21) Antibody	E011007-1	50μg/50μl	IHC, WB,IF	Human, Mouse, Rat	Rabbit
GSK3β(Phospho-Ser9) Antibody	E011002-1	50μg/50μl	IHC, WB,IF	Human, Mouse, Rat	Rabbit
Src (Phospho-Tyr529) Antibody	E011153-1	50μg/50μl	IHC, WB	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.

CTNNB1 protein is part of a complex of proteins that constitute adherens junctions (AJs). AJs are necessary for the creation and maintenance of epithelial cell layers by regulating cell growth and adhesion between cells. The encoded protein also anchors the actin cytoskeleton and may be responsible for transmitting the contact inhibition signal that causes cells to stop dividing once the epithelial sheet is complete. Finally, this protein binds to the product of the APC gene, which is mutated in adenomatous polyposis of the colon. Mutations in this gene are a cause of colorectal cancer (CRC), pilomatrixoma (PTR), medulloblastoma (MDB), and ovarian cancer. Three transcript variants encoding the same protein have been found for this gene. Involved in the regulation of cell adhesion and in signal transduction through the Wnt pathway.

Glycogen synthase kinase 3-alpha (**GSK**3A; EC {2.7.1.37}) is a multifunctional protein serine kinase, homologous to Drosophila 'shaggy' (zeste-white3) and implicated in the control of several regulatory proteins including glycogen synthase (see GYS1, {138570}) and transcription factors (e.g., JUN, {165160}). It also plays a role in the WNT ({164820}) and PI3K (see PIK3CG, {601232}) signaling pathways. Implicated in the hormonal control of several regulatory proteins including glycogen synthase, MYB and the transcription factor JUN (By similarity).

GSK3B protein is a serine-threonine kinase, belonging to the glycogen synthase kinase subfamily. It is involved in energy metabolism, neuronal cell development, and body pattern formation. Polymorphisms in this gene have been implicated in modifying risk of Parkinson disease, and studies in mice show that overexpression of this gene may be relevant to the pathogenesis of Alzheimer disease. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. Participates in the Wnt signaling pathway. Implicated in the hormonal control of several regulatory proteins including glycogen synthase, MYB and the transcription factor JUN. Phosphorylates JUN at sites proximal to its DNA-binding domain, thereby reducing its affinity for DNA. Phosphorylates MUC1 in breast cancer cells, and decreases the interaction of MUC1 with CTNNB1/beta-catenin. Phosphorylates CTNNB1/beta-catenin.

Glycogen synthase kinase 3 (GSK-3), a serine-threonine kinase with two isoforms (alpha and beta), was originally discovered as a key enzyme in glycogen metabolism. GSK-3 was subsequently shown to function in cell division, proliferation, motility and survival. GSK-3 plays a role in a number of pathological conditions including cancer and diabetes and is increasingly seen as an important component of neurological diseases. GSK-3 phosphorylates tau and presenilin-1, which are involved in the development of Alzheimer's disease. Both isoforms of GSK-3 are ubiquitously expressed, although particularly high levels of GSK-3beta are found in the brain where it is involved in synaptic plasticity, possibly via regulation of NMDA receptor trafficking. GSK-3 phosphorylates over 40 different substrates including signaling proteins, transcription factors and

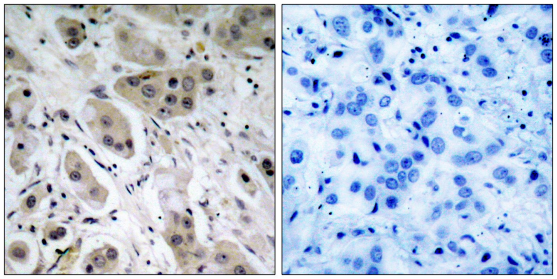
structural proteins, and is part of the signal transduction cascade of a large number of growth factors and cytokines. The activity of GSK is regulated by phosphorylation (Akt, S6K, RSK, PKA and PKC), dephosphorylation (PP1 and PP2A), and by binding to protein complexes (with beta-catenin, axin, CK1 and the APC complex).

SRC may play a role in the regulation of embryonic development and cell growth. The protein encoded by this gene is a tyrosine-protein kinase whose activity can be inhibited by phosphorylation by c-**SRC** kinase. Mutations in this gene could be involved in the malignant progression of colon cancer. Two transcript variants encoding the same protein have been found for this gene. Src kinases consist of eight non-receptor tyrosine kinases (Src, Fyn, Yes, Lck, Lyn, Hck, Fgr and Blk) that interact with the intracellular domains of growth factor/cytokine receptors, GPCRs and integrins. Members of the Src kinase family have a very similar domain structure with a high degree of homology in the SH1 (catalytic), linker, SH2 (p-Tyr binding), SH3 (protein-protein interaction) and SH4 (membrane association) domains. c-Src, Fyn and Yes are ubiquitously expressed, although high levels of c-Src are found in platelets, neural tissue and osteoclasts. For c-Src, autophosphorylation of Tyr418 and dephosphorylation of Tyr530 is required to switch the kinase from the inactive closed formation to the active open formation. c-Src can be inactivated by two kinases, c-Src kinase (CSK) and CSK homologous kinase (CHK), both of which phosphorylate Tyr530 of c-Src. The activity of the Src kinase family can also be regulated by phosphatases (e.g. SHP1), binding to adaptor proteins (e.g. Cbp) and proteasomal degradation. Src kinases are key upstream mediators of both the PI 3-K and MAPK signaling pathways, and have been shown to have important roles in cell proliferation, migration and survival.

Akt (Phospho-Ser473) Antibody

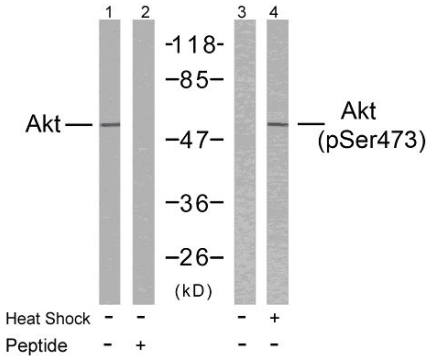
E011054

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).

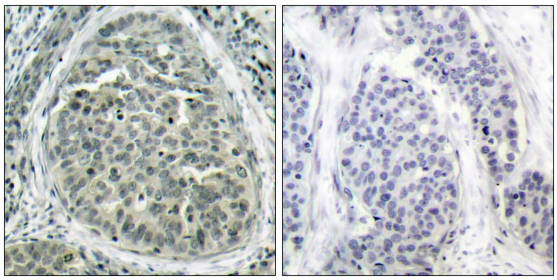


Western blot analysis of extract from HeLa cells untreated or treated with heat shock using Akt (Ab-473) antibody (E021054, Lane 1 and 2) and Akt (phospho-Ser473) antibody (E011054, Lane 3 and 4).

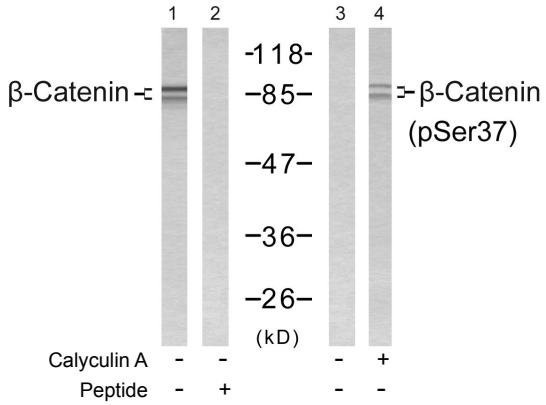
E011219

β-Catenin (Phospho-Ser37) Antibody

Catalog Number: E011219-1, E011219-2
Amount: 50μg/50μl, 100μg/100μl
Swiss-Prot No. : P35222
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 β-Catenin around the phosphorylation site of serine 37 (I-H-S^{P}-G-A).
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: β-Catenin (phospho-Ser37) antibody detects endogenous levels of β-Catenin only when phosphorylated at serine 37.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100
References:
Novak A, et al. (1998) Proc Natl Acad Sci U S A; 95(8): 4374-4379
Marin O, et al. (2003) Proc Natl Acad Sci U S A; 100(18): 10193-10200
Okamura H, et al. (2004) Mol Cell Biol; 24(10): 4184-4195
Xing Y, et al. (2003) Genes Dev; 17(22): 2753-2764
Barth AI, et al. (1999) Proc Natl Acad Sci U S A; 96(9): 4947-4952



Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue, using β-Catenin (phospho-Ser37) antibody (E011219).

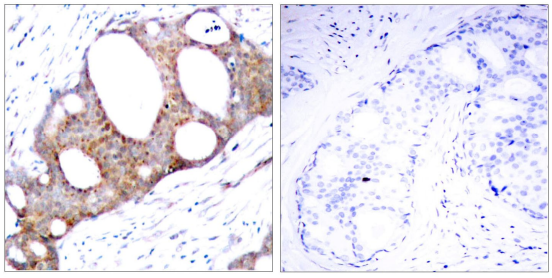


Western blot analysis of extract from SW 626 cells, using β-Catenin (Ab-37) antibody (E021212, Lane 1 and 2) and β-Catenin (phospho-Ser37) antibody (E011219, Lane 3 and 4).

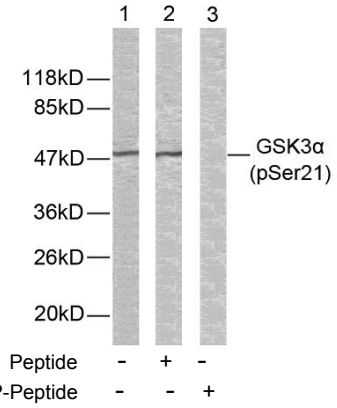
GSK3α (Phospho-Ser21) Antibody

E011007

Catalog Number: E011007-1, E011007-2
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 GSK3α around the phosphorylation site of serine 21 (T-S-S^{P}-F-A).
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: GSK3α (phospho-Ser21) antibody detects endogenous levels of GSK3α only when phosphorylated at serine 21.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100 IF: 1:100-200
Swiss-Prot No. : P49840
References:
Barry FA, et al. (2003) FEBS Lett. 553(1-2): 173-178.
Koivisto L, et al. (2003) J Cell Sci. 116(Pt 18): 3749-3760.
Welsh G I, et al. (1996) Trends Cell Biol. 6:274-279.
Srivastava A K, et al. (1998) Mol. Cell. Biochem. 182: 135-141.



Immunohistochemical analysis of paraffin- embedded human breast carcinoma tissue using GSK3α (phospho-Ser21) antibody (E011007).

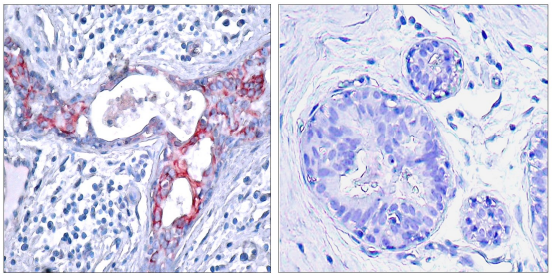


Western blot analysis of extracts from ovary cancer cells using GSK3α (phospho-Ser21) antibody (E011007).

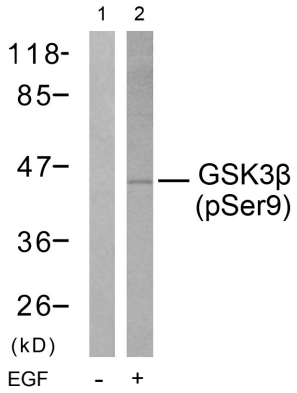
GSK3β (Phospho-Ser9) Antibody

E011002

Catalog Number: E011002-1, E011002-2
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 GSK3β around the phosphorylation site of serine 9 (T-T-S^P-F-A).
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: GSK3β (phospho-Ser9) antibody detects endogenous levels of GSK3β only when phosphorylated at serine 9.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100 IF: 1:100-200
Swiss-Prot No. : P49841
References:
Fan G, et al. (2003) J Biol Chem. 278(52): 52432-52436.
Barry FA, et al. (2003) FEBS Lett. 553(1-2): 173-178.
Welsh, et al. (1996) Trends Cell Biol. 6: 274-279.
Srivastava A K, et al. (1998) Mol Cell Biochem. 182: 135-141.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using GSK3β (phospho-Ser9) antibody (E011002).

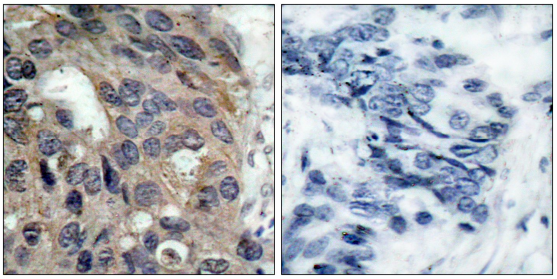


Western blot analysis of extracts from HeLa cells using GSK3β (phospho-Ser9) antibody (E011002).

Src (Phospho-Tyr529) Antibody

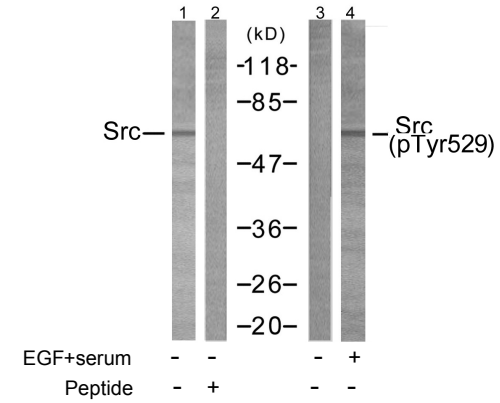
E011153

Catalog Number: E011153-1, E011153-2
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 Src around the phosphorylation site of tyrosine 529 (P-Q-Y^{P}-Q-P).
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: Src (phospho-Tyr529) antibody detects endogenous levels of Src only when phosphorylated at tyrosine 529.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100
Swiss-Prot No. : P12931
References:
Pyper J.M., (1985) Mol. Cell. Biol. 5:831-838
Pyper J.M.(1990) Mol. Cell. Biol. 10:2035-2040
Xu W., (1997).Nature 385:595-602
Benes C.H., (2005) Cell 121:271-280



P-Peptide - +

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using Src (phospho-Tyr529) antibody (E011153).



Western blot analysis of extracts from 293 cells using Src (Ab-529) antibody (E021168, Lane1 and 2) and Src (phospho-Tyr529) antibody (E011153, Lane 3 and 4).