



Phospho-Integrin Pathway Sampler Kit

E051026

Kits Includes	Cat.	Quantity	Application	Reactivity	Source
Integrin $\beta 3$ (Phospho-Tyr773) Antibody	E011060-1	50 μ g/50 μ l	IHC, WB	Human, Mouse	Rabbit
Paxillin (Phospho-Tyr31) Antibody	E011201-1	50 μ g/50 μ l	WB	Human	Rabbit
FAK (Phospho-Tyr861) Antibody	E011059-1	50 μ g/50 μ l	WB	Human, Mouse, Rat	Rabbit
Src(Phospho-Tyr418) Antibody	E011091-1	50 μ g/50 μ l	IHC, WB	Human, Mouse, Rat	Rabbit
Stathmin 1 (Phospho-Ser37) Antibody	E011225-1	50 μ g/50 μ l	IHC, WB	Human, Mouse, Rat	Rabbit

Integrins are integral cell-surface proteins composed of an alpha chain and a beta chain. A given chain may combine with multiple partners resulting in different integrins. Integrin beta 3 is found along with the alpha IIb chain in platelets. Integrins are known to participate in cell adhesion as well as cell-surface mediated signalling. Integrin alpha-V/beta-3 is a receptor for cytotactin, fibronectin, laminin, matrix metalloproteinase-2, osteopontin, osteomodulin, prothrombin, thrombospondin, vitronectin and von Willebrand factor. Integrin alpha-IIb/beta-3 is a receptor for fibronectin, fibrinogen, plasminogen, prothrombin, thrombospondin and vitronectin. Integrins alpha-IIb/beta-3 and alpha-V/beta-3 recognize the sequence R-G-D in a wide array of ligands. Integrin alpha-IIb/beta-3 recognizes the sequence H-H-L-G-G-G-A-K-Q-A-G-D-V in fibrinogen gamma chain. Following activation integrin alpha-IIb/beta-3 brings about platelet/platelet interaction through binding of soluble fibrinogen. This step leads to rapid platelet aggregation which physically plugs ruptured endothelial surface. In case of HIV-1 infection, the interaction with extracellular viral Tat protein seems to enhance angiogenesis in Kaposi's sarcoma lesions.

Integrins are ubiquitously expressed adhesion molecules. They are cell-surface receptors that exist as heterodimers of alpha and beta subunits. At physiological conditions, integrins are highly glycosylated and contain a Ca^{2+} or Mg^{2+} ion, which is essential for ligand binding. Integrin receptors are critical for cell attachment to the extracellular matrix (ECM) and this is mediated through integrin-fibronectin, -vitronectin, -collagen and -laminin interactions. Intracellularly, integrins form adhesion complexes with proteins including talin, vinculin, paxillin and alpha-actinin. They also regulate kinases, such as focal adhesion kinase and Src family kinases, to mediate attachment to the actin cytoskeleton. Integrins also have a significant role in cell signaling and can activate protein kinases involved in the regulation of cell growth, division, survival, differentiation, migration and apoptosis. Glycoprotein II/IIIb (alphaIIbbeta3) is an integrin receptor found on the surface of platelets. It is involved in the cross-linking of platelets with fibrin, and so has a vital role in blood clot formation. Cytoskeletal protein involved in actin-membrane attachment at sites of cell adhesion to the extracellular matrix (focal adhesion). Phosphorylated on tyrosine residues during integrin-mediated cell adhesion, embryonic development, fibroblast transformation and following stimulation of cells by mitogens.

PTK2 gene encodes a cytoplasmic protein tyrosine kinase which is found concentrated in the focal adhesions that form between cells growing in the presence of extracellular matrix constituents. The encoded protein is a member of the FAK subfamily of protein tyrosine kinases but lacks significant sequence similarity to kinases from other subfamilies. Activation of this gene may be an important early step in cell growth and intracellular signal transduction pathways triggered in response to certain neural peptides or to cell interactions with the extracellular matrix. At least four transcript variants encoding four different isoforms have been found for this gene, but the full-length natures of only two of them have been determined. Non-receptor protein-tyrosine kinase implicated in signaling pathways involved in cell motility, proliferation and apoptosis. Activated by tyrosine-phosphorylation in response to either integrin clustering induced by cell adhesion or antibody cross-linking, or via G-protein coupled receptor (GPCR) occupancy by ligands such as bombesin or lysophosphatidic acid, or via LDL receptor occupancy. Plays a potential role in oncogenic transformations resulting in increased kinase activity.

Focal adhesion kinase (FAK), a non-receptor tyrosine kinase, is the first intracellular step in the signal transduction cascade initiated by the attachment of an integrin to the extracellular matrix at points known as focal adhesions. Therefore, FAK plays a key role in cellular migration and motility. FAK has 3 functional domains: a focal adhesion targeting domain (FAT), a catalytic domain and a FERM domain, which mediates interactions with the cytoplasmic domains of integrins and growth factor receptors. FAK has multiple phosphorylation sites that are required for binding to adaptor proteins containing SH2 domains (e.g. Src and SH3 domains (e.g. CAS, GRAF)). Key amongst these phosphorylation sites is Tyr397, which is important for the interaction of FAK with downstream signaling molecules such as PI 3-K, PLCgamma and Rho kinase. Overexpression of FAK has been associated with several types of cancer.

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. Phosphorylated on Tyr-530 by c-Src kinase (CSK). The phosphorylated form is termed

pp60c-src. The phosphorylated tail interacts with the SH2 domain thereby repressing kinase activity.

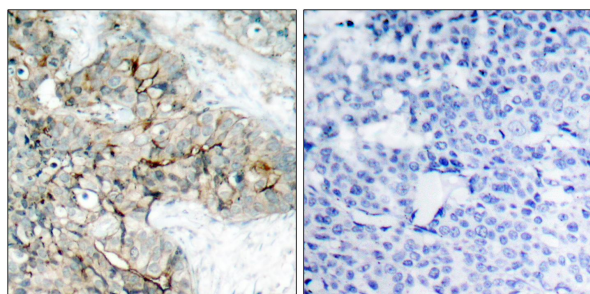
STMN1 gene belongs to the stathmin family of genes. It encodes a ubiquitous cytosolic phosphoprotein proposed to function as an intracellular relay integrating regulatory signals of the cellular environment. The encoded protein is involved in the regulation of the microtubule filament system by destabilizing microtubules. It prevents assembly and promotes disassembly of microtubules. Multiple transcript variants encoding different isoforms have been found for this gene. Involved in the regulation of the microtubule (MT) filament system by destabilizing microtubules. Prevents assembly and promotes disassembly of microtubules. Phosphorylation at Ser-16 may be required for axon formation during neurogenesis. Involved in the control of the learned and innate fear (By similarity). Many different phosphorylated forms are observed depending on specific combinations among the sites which can be phosphorylated. MAPK is responsible for the phosphorylation of stathmin in response to NGF. Phosphorylation at Ser-16 seems to be required for neuron polarization (By similarity). Phosphorylation at Ser-63 reduces tubulin binding 10-fold and suppresses the MT polymerization inhibition activity.



Integrin $\beta 3$ (Phospho-Tyr773) Antibody

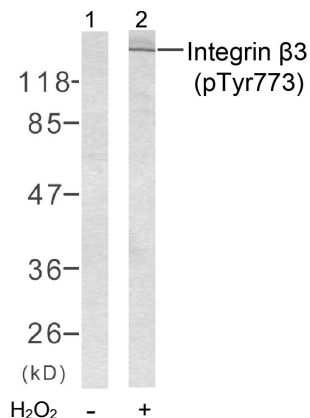
E011060

- Catalog Number:** E011060-1, E011060-2
- Amount:** 50 μ g/50 μ l, 100 μ g/100 μ l
- Form of Antibody:** Rabbit IgG in phosphate buffered saline (without Mg²⁺ and Ca²⁺), 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 Integrin $\beta 3$ around the phosphorylation site of tyrosine 773 (P-L-Y^P-K-E).
- 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:** Integrin $\beta 3$ (phospho-Tyr773) antibody detects endogenous levels of Integrin $\beta 3$ only when phosphorylated at tyrosine 773.
- Reactivity:** Human, Mouse
- Applications:** WB: 1:500~1:1000 IHC: 1:50~1:100
- Swiss-Prot No. :** P05106
- References:** Chandhoke SK, et al. (2004) J Cell Sci.117 (Pt 8): 1431-1441.
Datta A, et al. (2002) J Biol Chem.277 (6): 3943-3949.
Law DA, et al. (1996) J Biol Chem.271 (18): 10811-10815.



P-Peptide - +

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using Integrin $\beta 3$ (phospho-Tyr773) antibody.



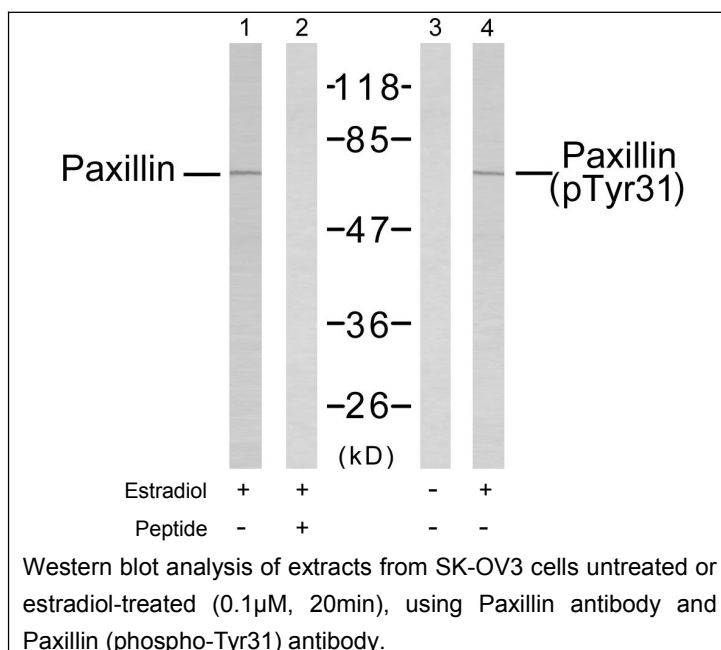
Western blot analysis of extracts from HL-60 cells using Integrin $\beta 3$ (phospho-Tyr773) antibody.



Paxillin (Phospho-Tyr31) Antibody

E011201

- Catalog Number:** E011201-1, E011201-2
- Amount:** 50µg/50µl, 100µg/100µl
- Swiss-Prot No. :** P49023
- All Names:** PAX-1, PXN, Paired box protein Pax-1, paxillin, paxillin alpha
- All Sites:** Human: Tyr31
- Swiss-Prot No. :** P49023
- All names:** Paxillin, PAXI, PXN, Paired box protein Pax-1, PAX-1
- Form of Antibody:** Rabbit IgG in phosphate buffered saline (without Mg²⁺ and Ca²⁺), 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 Paxillin around the phosphorylation site of tyrosine 31 (T-P-Y^P-S-Y).
- 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:** Paxillin (phospho-Tyr31) antibody detects endogenous levels of Paxillin only when phosphorylated at tyrosine 31.
- Reactivity:** Human
- Applications:** WB: 1:500~1:1000
- References:** Davidson D, et al. (2001) EMBO J 20(13): 3414-3426.
Fleming I, et al. (1999) Proc Natl Acad Sci U S A 96(3): 1123-1128.
Goldberg MB. (2001) Microbiol Mol Biol Rev 65(4): 595-626.
Kook S, et al. (2000) Mol Biol Cell 11(3): 929-939.





FAK (Phospho-Tyr861) Antibody

E011059

Catalog Number: E011059-1, E011059-2

Amount: 50µg/50µl, 100µg/100µl

Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg²⁺ and Ca²⁺), 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 FAK around the phosphorylation site of tyrosine 861 (H-I-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.

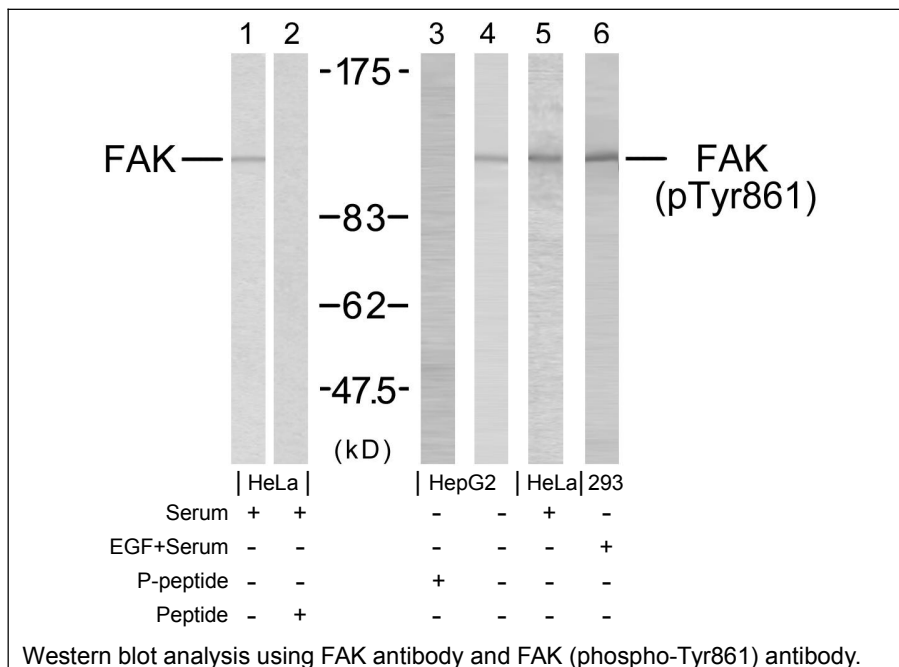
Specificity/Sensitivity: FAK (phospho-Tyr861) antibody detects endogenous levels of FAK only when phosphorylated at tyrosine 861.

Reactivity: Human, Mouse, Rat

Applications: WB: 1:500~1:1000

Swiss-Prot No. : Q05397

References: Shi Q, et al. (2003) Mol Biol Cell; 14(10): 4306-15.
Vadlamudi RK, et al. (2003) FEBS Lett; 543(1-3): 76-80.
Eliceiri BP, et al. (2002) J Cell Biol Apr 01; 157(1): 149-60.
Abu-Ghazaleh R, (2001) et al. Biochem J; 360(Pt 1): 255-64.

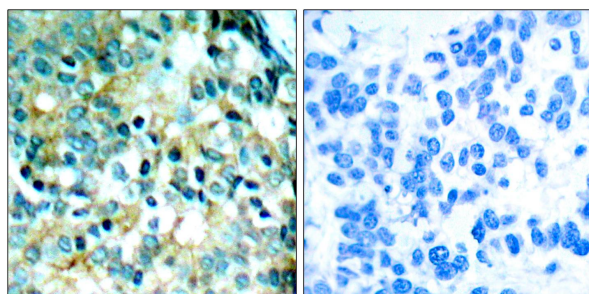




Src (Phospho-Tyr418) Antibody

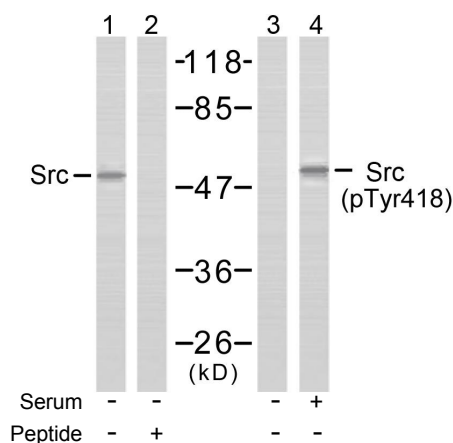
E011091

- Catalog Number:** E011091-1, E011091-2
- Amount:** 50µg/50µl, 100µg/100µl
- Form of Antibody:** Rabbit IgG in phosphate buffered saline (without Mg²⁺ and Ca²⁺), 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 418 (N-E-Y^P-T-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 chromatography using non-phosphopeptide corresponding to the phosphorylation site.
- Specificity/Sensitivity:** Src (phospho-Tyr418) antibody detects endogenous levels of Src only when phosphorylated at tyrosine 418.
- Reactivity:** Human, Mouse, Rat
- Applications:** WB: 1:500~1:1000 IHC: 1:50~1:100
- Swiss-Prot No. :** P12931
- References:** Dufilho M, et al. (2005) J Biol Chem. 280(43):35999-36006.
 Sanders MA, et al. (2005) J Biol Chem. 280(25): 23516-23522.
 Murugappan S, et al. (2005) Blood. 106(2):550-557.
 Gu JJ, et al. (2005) Blood. 105(8): 3270-3277.



P-Peptide - +

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using Src (phospho-Tyr418) antibody.



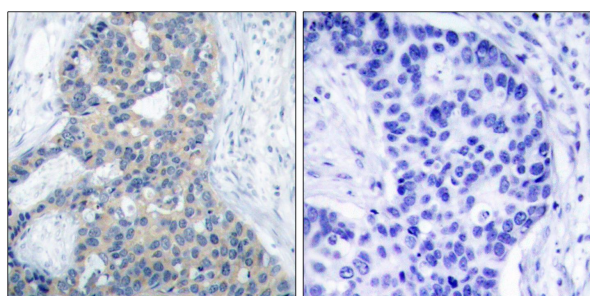
Western blot analysis of extracts from COLO205 cells using Src antibody and Src (phospho-Tyr418) antibody.



Stathmin 1 (Phospho-Ser37) Antibody

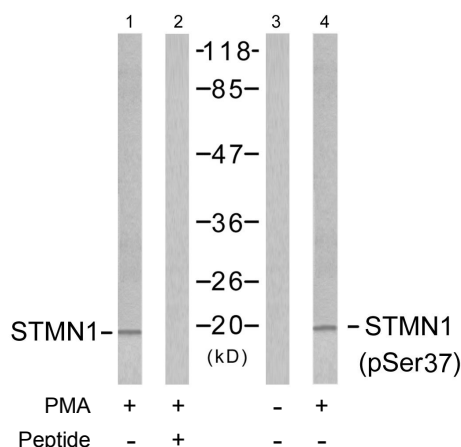
E011225

- Catalog Number:** E011225-1, E011225-2
- Amount:** 50µg/50µl, 100µg/100µl
- Swiss-Prot No. :** P16949
- All Names:** stathmin 1, LAG, LAP18, Leukemia-associated gene protein, Leukemia-associated phosphoprotein p18, Metablastin, Oncoprotein 18, Op18, PR22, Phosphoprotein p19, Pr22 protein, Prosolin, STN1, pp17, pp19, stathmin
- All Sites:** Human: Ser37; Mouse: Ser37; Rat: Ser37
- 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 Stathmin 1 around the phosphorylation site of serine 37 (P-L-S^P-P-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:** Stathmin 1 (phospho-Ser37) antibody detects endogenous levels of Stathmin 1 only when phosphorylated at serine 37.
- Reactivity:** Human, Mouse, Rat
- Applications:** WB: 1:500~1:1000 IHC: 1:50~1:100
- References:** Wang KK, et al. (1991) *Biochem J* 279(Pt 2): 537-544.
Sekimoto T, et al. (2004) *EMBO J* 23(9): 1934-1942.
Doye V, et al. (1992) *Biochem J* 287(Pt 2): 549-554.
Larsson N, et al. (1999) *Mol Cell Biol* 19(3): 2242-2250.



P-Peptide - +

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using Stathmin 1 (phospho-Ser37) antibody.



Western blot analysis of extracts from Jurkat cells untreated or treated with PMA (1ng/ml, 15min), using Stathmin 1 antibody and Stathmin 1 (phospho-Ser37) antibody.