Datasheet for ABIN968643

anti-FAK antibody (pTyr397)

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Overview

Quantity:	50 µg
Target:	FAK (PTK2)
Binding Specificity:	pTyr397
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This FAK antibody is un-conjugated
Application:	Western Blotting (WB), BioImaging (BI)

Product Details

Immunogen:	Human FAK (pY397) Peptide
Clone:	14-FAK
Isotype:	IgG1
Characteristics:	1. Since applications vary, each investigator should titrate the reagent to obtain optimal results. 2. Please refer to us for technical protocols. 3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance. 4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

Product Details

	5. Source of all serum proteins is from USDA inspected abattoirs located in the United States. 6. Triton is a trademark of the Dow Chemical Company.
Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Target Details

Target:	FAK (PTK2)
Alternative Name:	FAK (PTK2 Products)
Background:	Focal Adhesion Kinase (FAK) is a cytoplasmic tyrosine kinase that colocalizes with integrins in focal adhesions. This cellular localization is directed by a 125 amino acid sequence at the C-terminus called the Focal Adhesion Targeting sequence (FAT). The binding of extracellular matrix ligands to integrins triggers autophosphorylation at Tyr-397, and activation of FAK through phosphorylation of Tyr residues (Tyr-576 and Tyr577) in the kinase domain activation loop. For example, cell adhesion to a fibronectin substratum involves concurrent activation of Src and phosphorylation of the FAK activation loop. In addition, phosphorylation of other Tyr residues (Tyr-925, and Tyr-861) creates binding sites for SH2 domains of intracellular signaling molecules such as Src, PI3 kinase, and Grb2. FAK's ability to bind numerous structural and signaling proteins via a variety of interactions is important for FAK activation level, and for FAK interaction with a variety of substrates localized to sites of cell adhesion. Thus, FAK activity is regulated by a complex set of phosphorylation sites, and this phospho-regulation could be important for cell motility, cell growth, cytoskeletal organization, and adhesion-dependent cell survival. Synonyms: Focal Adhesion Kinase (pY397)
Molecular Weight:	116-125 kDa
Pathways:	Response to Growth Hormone Stimulus, CXCR4-mediated Signaling Events, Smooth Muscle Cell Migration, Signaling of Hepatocyte Growth Factor Receptor, VEGF Signaling

Application Details

Application Notes:	Bioimaging 1. Seed the cells in appropriate culture medium at ~10,000 cells per well in an 96-well Imaging Plate and culture overnight. 2. Remove the culture medium from the wells, and fix the cells by adding 100 myl of Fixation Buffer to each well. Incubate for 10 minutes at room temperature (RT).

Application Details

	3. Remove the fixative from the wells, and permeabilize the cells using either 90% methanol, or Triton™ X-100: a. Add 100 myl of -20°C 90% methanol to each well and incubate for 5 minutes at RT. OR b. Add 100 myl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT. 4. Remove the permeabilization buffer, and wash the wells twice with 100 myl of 1× PBS. 5. Remove the PBS, and block the cells by adding 100 myl of to each well. Incubate for 30 minutes at RT. 6. Remove the blocking buffer and add 50 myl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT. 7. Remove the primary antibody, and wash the wells three times with 100 myl of 1× PBS. 8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 myl to each well, and incubate in the dark for 1 hour at RT. 9. Remove the second step reagent, and wash the wells three times with 100 myl of 1× PBS. 10. Remove the PBS, and counter-stain the nuclei by adding 200 myl per well of 2 myg/ml Hoechst 33342 in 1× PBS to each well at least 15 minutes before imaging. 11. View and analyze the cells on an appropriate imaging instrument.
Comment:	Related Products: ABIN968536, ABIN968630, ABIN967389, ABIN967736
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	250 µg/mL
Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C
Storage Comment:	Store undiluted at -20°C.

Publications

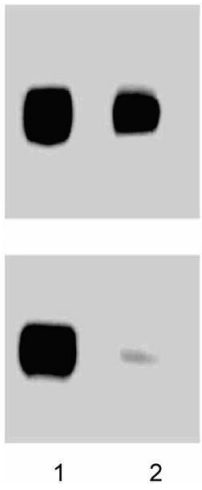
Product cited in:	McLean, Fincham, Frame: "v-Src induces tyrosine phosphorylation of focal adhesion kinase independently of tyrosine 397 and formation of a complex with Src." in: **The Journal of biological chemistry**, Vol. 275, Issue 30, pp. 23333-9, (2000) (PubMed).

Publications

Ruest, Roy, Shi, Mernaugh, Hanks: "Phosphospecific antibodies reveal focal adhesion kinase activation loop phosphorylation in nascent and mature focal adhesions and requirement for the autophosphorylation site." in: **Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research**, Vol. 11, Issue 1, pp. 41-8, (2000) (PubMed).

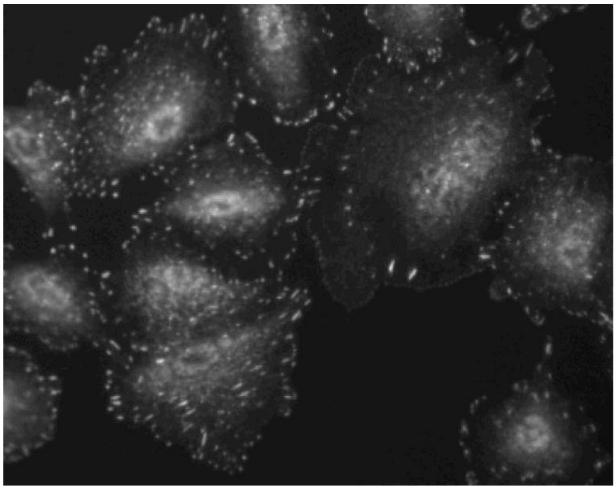
Calalb, Zhang, Polte, Hanks: "Focal adhesion kinase tyrosine-861 is a major site of phosphorylation by Src." in: **Biochemical and biophysical research communications**, Vol. 228, Issue 3, pp. 662-8, (1997) (PubMed).

Images



Western Blotting

Image 1. Western blotting for human FAK (pY397). Human endothelial cells were treated with 1 mM pervanadate, a general inhibitor of protein tyrosine phosphotases, for 15 minutes at 37°C then either left untreated (lane 1) or treated (lane 2) with 50 µg/ml alkaline phosphatase for 30 minutes at 37°C. The top panel was probed with mouse anti-FAK antibody (ABIN967736) and the bottom panel was probed with the mouse anti-human FAK (pY397) antibody at a 1:1000 dilution. The target band in each panel may be observable in a range of 116-125 kD.



Immunofluorescence

Image 2. Immunofluorescent staining of A549 cells. A549 cells (ATCC CCL-185) were seeded in a 96-well imaging plate at ~ 10,000 cells per well. After overnight incubation, cells were stained using the Triton™ X-100 fix/perm protocol and the mouse anti-human FAK (pY397) antibody. The second step reagent was Alexa Fluor® 488 goat anti-mouse Ig (Invitrogen). Images were taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained U-2 OS (ATCC HTB-96) and HeLa (ATCC CCL-2) cells using

either the Triton™ X-100 or alcohol perm protocols.

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