Bioimaging Certified Reagent

Technical Data Sheet

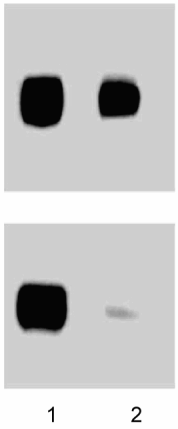
Purified Mouse Anti-Human FAK (pY397)

Product Information

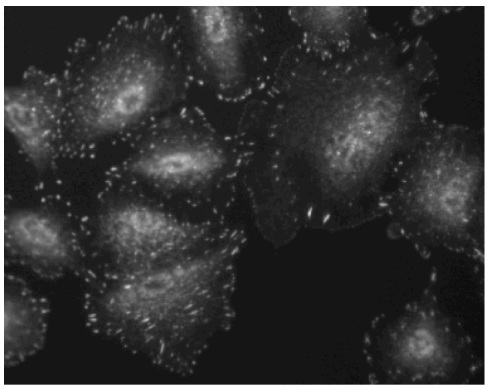
Material Number:	**611722**
Alternate Name:	Focal Adhesion Kinase (pY397)
Size:	50 µg
Concentration:	250 µg/ml
Clone:	14/FAK(Y397)
Immunogen:	Human FAK (pY397) Peptide
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	116-125 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

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.



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 (Cat. No. 610087) 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.*



Immunofluorescent staining of A549 cells. *A549 cells (Human lung carcinoma; ATCC CCL-185) were seeded in a BD Falcon™ 96-well imaging plate (Cat. No. 353219) at ~ 10,000 cells per well. After overnight incubation, cells were stained using the Triton-X 100 fix/perm protocol (see Recommended Assay Procedure) 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 Pathway 850 imager using a 20x objective. This antibody also stained U2OS and HeLa cells using either the Triton-X 100 or Methanol fix/perm protocols (see Recommended Assay Procedure).*

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Preparation and Storage

Store undiluted at -20° C.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Routinely Tested
Immunofluorescence	Tested During Development

Recommended Assay Procedure:
Western blot: Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml
Bioimaging: Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Bioimaging_Certified.shtml

Methanol Procedure for a 96 well plate:
Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:
Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Suggested Companion Products

Catalog Number	Name	Size	Clone
611450	Human Endothelial Cell Lysate	500 µg	(none)
611667	Human Endothelial + Pervanadate Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
610087	Purified Mouse Anti- FAK	50 µg	77/FAK
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. 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.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Calalb MB, Zhang X, Polte TR, Hanks SK. Focal adhesion kinase tyrosine-861 is a major site of phosphorylation by Src. *Biochem Biophys Res Commun.* 1996; 228(3):662-668.(Biology)
McLean GW, Fincham VJ, Frame MC. v-Src induces tyrosine phosphorylation of focal adhesion kinase independently of tyrosine 397 and formation of a complex with Src. *J Biol Chem.* 2000; 275(30):23333-23339.(Biology)
Ruest PJ, Roy S, Shi E, Mernaugh RL, Hanks SK. Phosphospecific antibodies reveal focal adhesion kinase activation loop phosphorylation in nascent and mature focal adhesions and requirement for the autophosphorylation site. *Cell Growth Differ.* 2000; 11(1):41-48.(Biology)