Technical Data Sheet

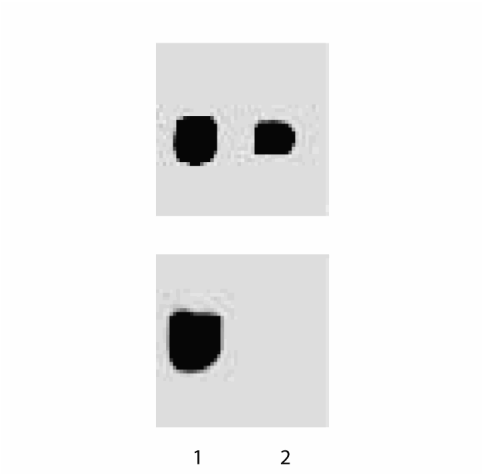
Purified Mouse Anti-Human FAK (pY397)

Product Information

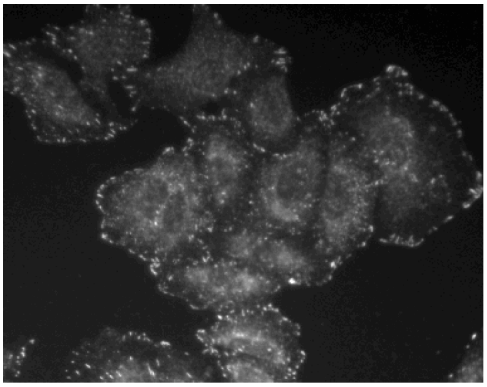
Material Number:	**611806**
Alternate Name:	Focal Adhesion Kinase (pY397)
Size:	50 µg
Concentration:	250 µg/ml
Clone:	18/FAK (pY397)
Immunogen:	Human FAK (pY397)
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	116-125 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, 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 "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 Tyr-577) 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 (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 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 and worked with both the Triton™ X-100 and alcohol 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	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl 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 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl 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 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl 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 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
610087	Purified Mouse Anti-FAK	50 µg	77/FAK
611667	Human Endothelial + Pervanadate Cell Lysate	500 µg	(none)
611450	Human Endothelial Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols 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.
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.

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)