Technical Data Sheet

Purified Mouse Anti-FAK

Product Information

Material Number:	**610087**
Alternate Name:	Focal Adhesion Kinase
Size:	50 µg
Concentration:	250 µg/ml
Clone:	77/FAK
Immunogen:	Chicken FAK aa. 354-533
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat, Dog, Chicken
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 and activation of FAK. This 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 has led to substantial speculation about its function. Although FAK's precise role has not been elucidated, proposed possibilities include regulating cell motility, cell growth, cytoskeletal organization, and adhesion-dependent cell survival.

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
Immunohistochemistry	Tested During Development
Immunoprecipitation	Tested During Development
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.

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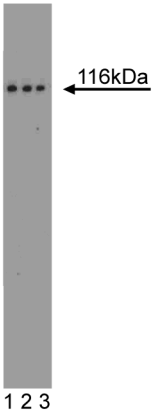
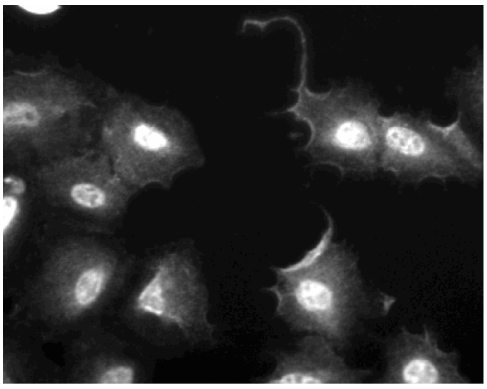


Western blot analysis of FAK on a A431 cell lysate (Human epithelial carcinoma; ATCC CRL-1555). *Lane 1: 2 µg/ml, lane 2: 1 µg/ml, lane 3: 0.5 µg/ml of the mouse anti- FAK antibody.*



Immunofluorescent staining of U2OS (ATCC HTB-96) cells. *Cells 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 alcohol perm protocol and the anti-FAK antibody. The second step reagent was FITC goat anti-mouse Ig (Cat. No. 554001). Images were taken on a BD Pathway™ 855 bioimaging system using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and HeLa (ATCC CCL-2) cells and worked with both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).*

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611447	A431 Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554655	Fixation Buffer	100 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

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