10 Old Barn Road • Lake Placid, NY 12946
Technical Support: T: 800 548-7853 • F: 518 523-4513
email: techserv@upstatebiotech.com
Sales Department: T: 800 233-3991 • F: 781 890-7738
Licensing Dept.: 800 310-4659
www.upstatebiotech.com

Certificate of Analysis

Anti-FAK, clone 4.47

(mouse monoclonal IgG_1)
Catalog # 05-537
Lot # 23207

Immunogen: GST fusion protein corresponding to residues 1-423 of human FAK (focal adhesion kinase). Clone 4.47.

Specificity: Recognizes and is specific for $p125^{FAK}$. Does not cross react with Pyk-2.

Species Cross-reactivity: Human, mouse and rat.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

Formulation: **200µg** of protein G purified mouse IgG_1 in **200µl** of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

Immunoblot Analysis: 0.2-1µg/ml of this lot detected FAK from a 3T3/A31 RIPA cell lysate. 0.2-1µg/ml of a previous lot detected FAK from PC-12, Hep-G2, SW 620 and Jurkat RIPA cell lysates.

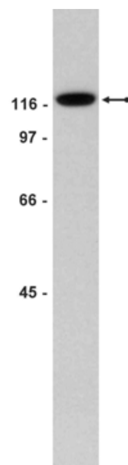
Included Positive Antigen Control: Catalog # 12-305, 3T3/A31 Cell Lysate. **Add 2.5µl of 2-mercaptoethanol per 100µl of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for minigels.

Immunoprecipitation: 5µg of a previous lot immuno-precipitated FAK from 250µg of 3T3/A31 RIPA cell lysate.

Additional Research Applications

Immunohistochemistry: This antibody is reported to detect FAK in human breast carcinoma sections.

Immunocytochemistry: This antibody is reported to show positive immunostaining for FAK in human breast carcinoma cells at 2.5-5µg/ml dilution.



Immunoblot Analysis
3T3/A31 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-FAK (0.2µg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates FAK.

General References:
Shen, Y. and M.D. Schaller, Mol. Biol. Cell **10**: 2507-2518, 1999.
van de Water, B., *et al.,* J. Biol. Chem. **274**: 13328-13337, 1999.

Application References:
Cance, W.G., *et al.,* Clin. Cancer Res. **6**: 2417-2423, 2000.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1μg/ml each aprotinin, leupeptin, pepstatin; 1mM Na_3VO_4; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 45 minutes to 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.2-1μg/ml of anti-FAK, clone 4.47,** diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:2000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 10 minutes.
8. Rinse the nitrocellulose in water for 30 minutes or longer.
9. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation Protocol

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1μg/μl total cell protein in a microcentrifuge tube with PBS.
2. Add **5μg of anti-FAK, clone 4.47,** to 250μg-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100μl (50μl packed beads) of washed Protein G agarose bead slurry (Catalog # 16-266).
5. Gently rock the reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50μl 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes. Collect the beads by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblot analysis can be performed on a sample of the supernatant.

Immunocytochemistry Protocol

1. Plate approximately 200μl of cell suspension into each well of a slide. Incubate 24 hours in a 37°C CO_2 incubator.
2. Wash the cells three times with PBS. Do not shake cells.
3. Add fix (3.7% formaldehyde) in PBS for 10 minutes at room temperature.
4. Wash the cells three times with PBS. Do not shake.
5. Permeabilized in 0.1% Triton-X100 in PBS for 3 minutes at room temperature.
6. Wash cells with PBS for 5 minutes at room temperature.
7. Cover cells with 400μl of 10% normal goat serum in PBS and incubate for 30 minutes at room temperature.
8. Wash the cells three times with PBS.
9. Incubate the cells with **2.5-5μg/ml anti-FAK, clone 4.47,** in PBS and incubate for 1 hour at room temperature.
10. Wash the cells three times, 5 minutes each with PBS.
11. Incubate the cells with a 1:100 dilution of goat anti-mouse IgG fluorescein conjugated secondary antibody in PBS for 45 minutes at room temperature.
12. Wash the cells three times with PBS.
13. Mount and examine the cells under a fluorescent microscope.