

Certificate of Analysis

Anti-FAK, clone 4.47

(mouse monoclonal IgG₁)

Catalog # 05-537

Lot # 29870

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.

Formulation: 200µg of protein G purified mouse IgG₁ in 200µl of 70% storage buffer (0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide) and 30% glycerol. Store at -20°C.

Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

**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.

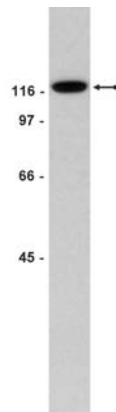
Included Positive Antigen Control: Catalog # 12-305, 3T3/A31 Cell Lysate. **Add 2.5µl of 2-mercaptoethanol per 100µl of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for minigels.

Immunoprecipitation: 5µg of a previous lot immunoprecipitated 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

Representative blot from a previous lot. 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.

Application References:

1. Cance, W.G., *et al.*, Clin. Cancer Res. **6**: 2417-2423, 2000.
2. Abrahamsen, H., *et al.*, J. Biol. Chem. **278**: 17170-7, 2003.

General References:

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

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 EDTA; 1mM PMSF; 1 μ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 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 **0.2-1 μ g/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 1 μ g/ μ l total cell protein in a microcentrifuge tube with PBS.
2. Add **5 μ g of anti-FAK, clone 4.47**, to 250 μ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 μ l (50 μ l 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 60 μ l 2X Laemmli sample buffer.
8. Store the beads frozen for future analysis or boil the beads for 5 minutes.
9. Collect the beads after boiling using a microcentrifuge pulse.
10. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.

Immunocytochemistry Protocol

1. Plate approximately 200 μ l of cell suspension into each well of a slide. Incubate 24 hours in a 37°C CO₂ 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 400 μ l 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 **2.5-5 μ g/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 in the dark, 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.