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cell signaling solutions
a Serologicals® Company

Certificate of Analysis

Anti-FAK
(rabbit polyclonal IgG)
Catalog # 06-543
Lot # 31701

Immunogen: pGEX-derived fusion protein containing residues 748-1053 of human FAK.

Specificity: Recognizes and is specific for human $p125^{FAK}$; does not cross-react with Pyk2.

Species Cross-reactivity: Human, mouse, rat and hamster **not** avian.

Formulation: **200µg** of protein A purified rabbit IgG in **200µl** of storage buffer (0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide). 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.**

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

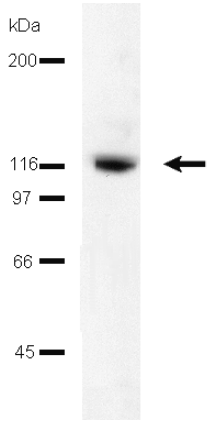
Immunoblot Analysis: 0.5-2µg/ml of this lot detected FAK in RIPA lysates from murine 3T3/A31 and previously in human A431 RIPA cell lysate.

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

Immunoprecipitation: 4µg of this lot immunoprecipitated FAK from 500µg of murine 3T3/A31 RIPA lysate.

Additional Research Applications

Immunocytochemistry: 10µg/ml of a previous lot showed positive immunostaining for Fak in Ref 52 cells fixed with 3.7% formaldehyde.



Immunoblot Analysis
Representative blot from a previous lot. 3T3/A31 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-FAK (1µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates FAK (125kDa).

Application References:
1. Ohtaki, T., *et al.,* Nature **411:** 613-616, 2001.
2. Zabe, M., *et al.,* J. Biol. Chem. **276:** 14704-14709, 2001.
3. Gervais, F.G., *et al.,* J. Biol. Chem. **273:** 17102-17108, 1998.

References:
4. Kanner, S., *et al.,* Proc. Natl. Acad. Sci. USA **87:** 3328, 1990.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 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 PBS-0.05% Tween 20 for 20 minutes at room temperature with constant agitation.
3. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 20 minutes at room temperature with constant agitation.
4. Incubate the nitrocellulose with **0.5-2μg/ml of anti-FAK,** diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
5. Wash the nitrocellulose twice with water.
6. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
7. Wash the nitrocellulose with water twice.
8. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
9. Rinse the nitrocellulose in 4-5 changes of water.
10. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation Protocol

1. Add **4μg of anti-FAK** and 60μl (30μl packed beads) of washed Protein A agarose bead slurry (Catalog # 16-125) to 500μl of PBS in a microcentrifuge tube.
2. Gently rock the reaction mixture at 4°C for 1 hour.
3. 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.
4. Dilute the cell lysate to roughly 1μg/μl total cell protein with PBS.
5. Add 500μg-1mg cell lysate to the reaction mixture.
6. Gently rock the reaction mixture at 4°C for 1 hour.
7. 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.
8. Resuspend the agarose beads in 70μl 2X Laemmli sample buffer.
9. Store the beads frozen for future analysis or boil the beads for 5 minutes.
10. Collect the beads after boiling using a microcentrifuge pulse.
11. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.

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.
3. Add fix (3.7% of formaldehyde) in PBS for 10 minutes at room temperature.
4. Wash the cells three times with PBS.
5. Permeabilize with 0.1% Triton-X100/PBS for 3 min at room temperature.
6. Wash the cells three times with PBS.
7. Cover cells with 400 μl of 1% BSA in PBS and incubate for 30 minutes at room temperature.
8. Wash the cells with three times with PBS.
9. Incubate the cells with **10μg/ml anti-FAK** in 1% BSA in PBS and incubate for 1 hour at room temperature.
10. Wash the cells three times with PBS.
11. Incubate the cells, in the dark with a 1:100 dilution of goat anti-rabbit IgG fluorescein conjugated secondary antibody in 1% BSA in PBS for 45 minutes at room temperature (keep in dark).
12. Wash the cells three times with PBS.
13. Mount and examine the cells under a fluorescent microscope.