

Certificate of Analysis

Anti-FAK

(rabbit polyclonal IgG)

Catalog # 06-543

Lot # 21819

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.

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

Formulation: 200mg of protein A purified rabbit IgG in 200ml 0.1M Tris-glycine, pH 7.4, 0.15M NaCl containing 0.05% sodium azide. Frozen solution.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

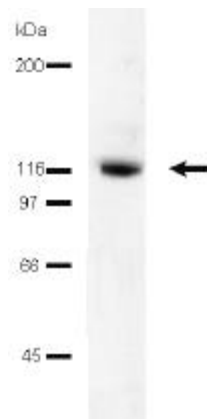
Immunoblot Analysis: 0.5-2µg/ml of this lot detected FAK in RIPA lysates from murine 3T3 and previously in human A431 RIPA cell lysate. Previous lots have detected an additional weak band at 110kDa but with shorter exposure times no additional band was detected.

Included Positive Antigen Control: Catalog # 12-305, 3T3 cell lysate. **Add 2.5ml of 2-mercaptoethanol/100ml 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 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 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).

References:

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 EGTA; 1mM PMSF; 1µg/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 PBS-0.05% Tween for 20 minutes at 20-25°C with constant agitation.
3. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in PBS (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
4. Incubate the nitrocellulose with **0.5-2mg/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. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1µg/µl total cell protein in a microcentrifuge tube with PBS.
2. Add **4mg of anti-FAK** to 500µg-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 A agarose bead slurry (Catalog # 16-125).
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 CO₂ 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 **10mg/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 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.