

Anti-Human Focal Adhesion Kinase (p125^{FAK})

(rabbit polyclonal IgG)

Catalog # 06-543

Lot # 16334

Immunogen: pGEX-derived fusion protein containing residues 748-1053 of human p125^{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: **200ng** of protein A purified rabbit IgG in **200nl** 0.1M Tris-glycine, pH 7.4, 0.15M NaCl containing 0.05% sodium azide. Frozen solution.

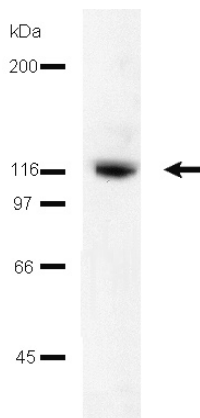
FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

Immunoblot Analysis: 1µg/ml of this lot detected p125^{FAK} in RIPA lysates from murine 3T3 (Catalog #12-305) and human A431 cells. An additional weak band was detected at 110kDa. Using shorter exposure times no additional bands are detected.

Included Positive Antigen Control: Catalog #12-305, 3T3 cell lysate. Use 20µg per lane for minigels.

Immunoprecipitation: 4µg of this lot immunoprecipitated p125^{FAK} from 500µg of murine 3T3 RIPA lysate.



Immunoblot Analysis

3T3 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Human Focal Adhesion Kinase (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 PBS containing 3% nonfat dry milk (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
4. Incubate the nitrocellulose with **1ng/ml of α-Human Focal Adhesion Kinase**, 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 α-rabbit IgG** linked to horseradish peroxidase, 1:3000 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 **4ng of α- Human Focal Adhesion Kinase** to 500µg-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100µl of washed Protein A agarose bead slurry (50µl packed beads).
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.