

Certificate of Analysis

Anti-phospho-FAK (Tyr576)

(rabbit antiserum)

Catalog # 07-157

Lot # 20262

Immunogen: Synthetic peptide corresponding to amino acids 571-582 of human FAK (MEDST[pY]YKASKG-C). The immunizing sequence is identical in mouse, rat, chicken and *Xenopus*.

Specificity: Recognizes FAK phosphorylated at Tyr576, Mr 125kDa.

Species Cross-reactivity: Human, mouse, and rat. Wide species cross-reactivity is expected due to conservation of immunizing sequence.

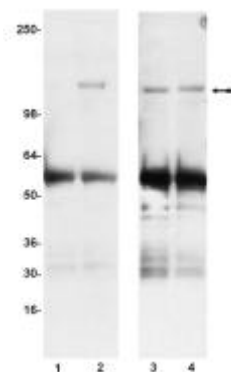
Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

Formulation: 200ml of rabbit antiserum with 0.05% sodium azide and 30% glycerol. Liquid at -20°.

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

Quality Control Testing

Immunoprecipitation/Immunoblot: A 1:500 dilution of this lot detected phosphorylated FAK which was immunoprecipitated using 4µg of polyclonal anti-FAK (Catalog # 06-543) from p60^{Src(527F)}-transformed Rat-2 cells but not control Rat-2 cells.



Immunoprecipitation/Immunoblot Analysis

FAK immunoprecipitates from control (Lanes 1 and 3) and p60^{Src(527F)}-transformed (Lanes 2 and 4) Rat2 cell lysates were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-FAK (Tyr576) (1:500; Lanes 1 and 2) or anti-FAK (Catalog # 06-543; Lanes 3 and 4). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates FAK (125kDa).

Additional Applications:

Immunocytochemistry: A 1:20 dilution of this antibody has been reported by an independent laboratory, to immunostain phospho-FAK in fibroblasts plated onto fibronectin-coated cover slips, fixed with 3.7% paraformaldehyde and permeabilized with 0.5% Triton X-100.¹

Application Reference:

1. Ruest, P.J., *et al.*, *Cell Growth Differ.* **11**: 41-48, 2000.

Immunoprecipitation/Immunoblot Protocol

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly $1\mu\text{g}/\mu\text{l}$ total cell protein in a microcentrifuge tube with PBS.
2. Add $4\mu\text{g}$ of anti-FAK (Catalog # 06-543) to $500\mu\text{g}$ - 1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding $100\mu\text{l}$ ($50\mu\text{l}$ 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 $14,000 \times g$), 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 $50\mu\text{l}$ 2X Laemmli sample buffer and boil for 5 minutes. Collect the beads by a microcentrifuge pulse and perform SDS-PAGE and immunoblot analysis on a sample of the supernatant.
8. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (TBS-MLK) for 30 minutes at $20\text{-}25^{\circ}\text{C}$ with constant agitation.
9. Incubate the nitrocellulose with a **1:500 dilution of anti-phospho-FAK (Tyr 576)**, diluted in freshly prepared TBS-MLK overnight with agitation at 4°C .
10. Wash the nitrocellulose twice with water.
11. 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 TBS-MLK for 1.5 hours at room temperature with agitation.
12. Wash the nitrocellulose with water twice.
13. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
14. Rinse the nitrocellulose in 4-5 changes of water.
15. Use detection method of choice (enhanced chemiluminescence was used).