

Anti-phospho-FAK (Tyr576)

(rabbit antiserum)

Catalog # 07-157

Lot # 28089

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.

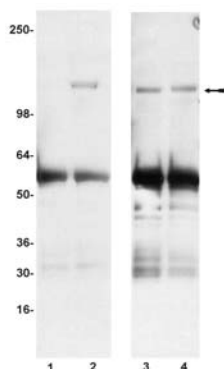
Formulation: 200 μ l of rabbit antiserum with 0.05% sodium azide and 30% glycerol. Liquid at -20°.

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.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

Immunoprecipitation/Immunoblot: A 1:300-1:500 dilution of this lot detected phosphorylated FAK which was immunoprecipitated using 4 μ g of polyclonal anti-FAK (Catalog # 06-543) from p60^{Src(527F)}-transformed Rat-2 cells but not control Rat-2 cells. Phospho-specificity was confirmed by dephosphorylation of the FAK protein with λ -phosphatase.



Immunoprecipitation/Immunoblot Analysis

Representative blot from a previous lot. 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. Add **4 μ g of anti-FAK** (Catalog # 06-543) and 60 μ l (30 μ l packed beads) of Protein A agarose bead slurry (Catalog # 16-125) to 500 μ l of PBS in a microcentrifuge tube.
2. Gently rock the reaction mixture at 4°C for one 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 1 μ g/ μ l total cell protein with PBS.
5. Add 500 μ g-1mg cell lysate to the reaction mixture.
6. Gently rock the reaction mixture at 4°C for 1-2 hours.
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 60 μ l 2X Laemmli sample buffer and boil for 5 minutes. Collect the beads by using a microcentrifuge pulse and perform SDS-PAGE and immunoblot analysis on a sample of the supernatant.
9. Block the blotted nitrocellulose in freshly prepared TBS containing 1% bovine serum albumin (BSA) and 0.1% Triton X-100 for 1 hour at room temperature with constant agitation.
10. Incubate the nitrocellulose in TBS containing 1% bovine serum albumin (BSA), 0.1% Triton X-100 and 2mM MnCl₂ and, where dephosphorylation of proteins is desirable, 400U/ml λ -phosphatase for 4 hours at room temperature, or overnight at 4°C.
11. Wash the nitrocellulose in PBS-0.1% Tween 20 for 3-5 minutes
12. Rinse the nitrocellulose in 4-5 changes of water.
13. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (Catalog # 20-200), (TBS-MLK) for 30 minutes at room temperature with constant agitation.
14. Incubate the nitrocellulose with a **1:300-1:500 dilution of anti-phospho-FAK (Tyr576)**, diluted in freshly prepared TBS-MLK overnight with agitation at 4°C.
15. Wash the nitrocellulose twice with water.
16. 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.
17. Wash the nitrocellulose with water twice.
18. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
19. Rinse the nitrocellulose in 4-5 changes of water.
20. Use detection method of choice (enhanced chemiluminescence was used).