



cell signaling solutions

## Certificate of Analysis

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### Anti-phospho-FAK (Tyr576)

(rabbit antiserum)

Catalog # 07-157

Lot # 23617

**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.

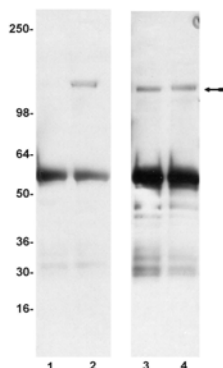
**Storage and Stability:** Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

**Formulation:** 200µl 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 1:500-1:1000 dilution of this lot detected phosphorylated FAK which was immunoprecipitated using 4µg of polyclonal anti-FAK (Catalog # 06-543) from p60<sup>Src(527F)</sup>-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<sup>Src(527F)</sup>-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.<sup>1</sup>

### 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 $\mu$ g/ $\mu$ l total cell protein in a microcentrifuge tube with PBS.
2. Add 4 $\mu$ g of anti-FAK (Catalog # 06-543) to 500 $\mu$ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 $\mu$ l (50 $\mu$ 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 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 50 $\mu$ 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 1% bovine serum albumin (BSA) and 0.1% Triton X-100 for 1 hour at room temperature with constant agitation.
9. Incubate the nitrocellulose in TBS containing 1% bovine serum albumin (BSA), 0.1% Triton X-100 and 2mM MnCl<sub>2</sub> and, where dephosphorylation of proteins is desirable, 400U/ml  $\lambda$ -phosphatase for 4 hours at room temperature, or overnight at 4°C.
10. Wash the nitrocellulose in PBS-0.1% Tween 20 for 3-5 minutes
11. Rinse the nitrocellulose in 4-5 changes of water.
12. 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.
13. Incubate the nitrocellulose with a **1:500-1:1000 dilution of anti-phospho-FAK (Tyr576)**, diluted in freshly prepared TBS-MLK overnight with agitation at 4°C.
14. Wash the nitrocellulose twice with water.
15. 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.
16. Wash the nitrocellulose with water twice.
17. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
18. Rinse the nitrocellulose in 4-5 changes of water.
19. Use detection method of choice (enhanced chemiluminescence was used).