

## Certificate of Analysis

### Anti-phospho-FAK (Tyr576)

(rabbit antiserum)

Catalog # 07-157

Lot # DAM1394797

**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:** 200µl 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.

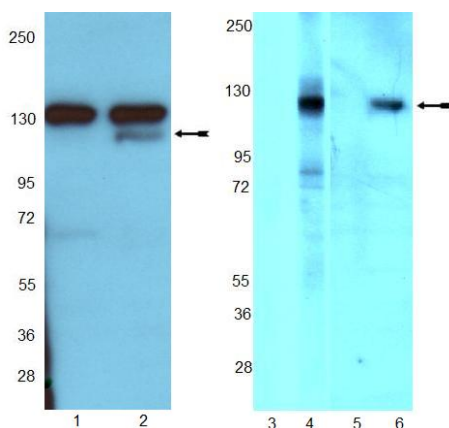
**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

### Quality Control Testing

**Immunoprecipitation/Immunoblot:** A 1:300-1:500 dilution of this lot detected phosphorylated FAK which was immunoprecipitated using 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.

### 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>



### Western Blot Analysis

Lanes 1 and 2 demonstrate untransformed and Src (527F)-transformed Rat2 cells (lanes 1 and 2, respectively) Lanes 3-6 demonstrate untreated (lanes 3 and 5) and pervanadate-treated (lanes 4 and 6) C2C12 (lanes 3 and 4) and HUVEC (lanes 5 and 6) cell lysate resolved by SDS-PAGE, transferred to PVDF, and probed with anti-phospho-FAK (Tyr576) (1:500) Proteins were visualized using a donkey anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates FAK (~125kDa).

### Application Reference:

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

### Immunoprecipitation/Immunoblot Protocol

1. Add **4µg of anti-FAK** (Catalog # 06-543) and 60µl (30µl packed beads) of Protein A agarose bead slurry (Catalog # 16-125) to 500µl 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 1µg/µl total cell protein with PBS.
5. Add 500µg-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 60µl 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 MnCl<sub>2</sub> 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).

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.