

## Certificate of Analysis

### Anti-FAK, clone 4.47

(mouse monoclonal IgG<sub>1</sub>)

Catalog # 05-537

Lot # 0701049384

**Immunogen:** GST fusion protein corresponding to residues 1-423 of human FAK (focal adhesion kinase). Clone 4.47.

**Specificity:** Recognizes and is specific for p125<sup>FAK</sup>. Does not cross react with Pyk-2. Depending on the antibody concentration or extracts used, the antibody may detect a non-specific band at approximately 50 kDa by western blot.

**Species Cross-reactivity:** Human, mouse and rat.

**Formulation:** 200 µg of protein G purified mouse IgG<sub>1</sub> in 200 µL of 70% storage buffer (0.014M phosphate buffer, pH 7.6, 0.175M NaCl, 0.07% sodium azide) and 30% glycerol. Store at -20°C.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment.

**Handling Recommendations:** Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

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

### Quality Control Testing

**Immunoblot Analysis:** 0.2-1.0 µg/mL of this lot detected FAK from a 3T3/A31 RIPA cell lysate. 0.2-1.0 µg/mL of a previous lot detected FAK from PC-12, Hep-G2, SW 620 and Jurkat RIPA cell lysates.

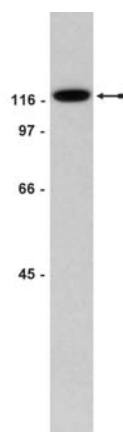
**Included Positive Antigen Control:** Catalog # 12-305, 3T3/A31 Cell Lysate. **Add 2.5 µL of 2-mercaptoethanol per 100 µL of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for minigels.

**Immunoprecipitation:** 5 µg of a previous lot immunoprecipitated FAK from 250 µg of 3T3/A31 RIPA cell lysate.

### Additional Research Applications

**Immunohistochemistry:** This antibody is reported to detect FAK in human breast carcinoma sections.

**Immunocytochemistry:** This antibody is reported to show positive immunostaining for FAK in human breast carcinoma cells at 2.5-5 µg/mL dilution.



#### Immunoblot Analysis

Representative blot from a previous lot. 3T3/A31 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-FAK (0.2 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates FAK.

### Application References:

1. Cance, W.G., *et al.*, Clin. Cancer Res. **6**: 2417-2423, 2000.
2. Abrahamsen, H., *et al.*, J. Biol. Chem. **278**: 17170-7, 2003.

### General References:

3. Shen, Y. and M.D. Schaller, Mol. Biol. Cell **10**: 2507-2518, 1999.
4. van de Water, B., *et al.*, J. Biol. Chem. **274**: 13328-13337, 1999.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1 $\mu$ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 45 minutes to 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.2-1 $\mu$ g/ml of anti-FAK, clone 4.47**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:2000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 10 minutes.
8. Rinse the nitrocellulose in water for 30 minutes or longer.
9. Use detection method of choice (enhanced chemiluminescence was used).

### Immunoprecipitation 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 **5 $\mu$ g of anti-FAK, clone 4.47**, to 250 $\mu$ 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 G agarose bead slurry (Catalog # 16-266).
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 60 $\mu$ l 2X Laemmli sample buffer.
8. Store the beads frozen for future analysis or boil the beads for 5 minutes.
9. Collect the beads after boiling using a microcentrifuge pulse.
10. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.

### Immunocytochemistry Protocol

1. Plate approximately 200 $\mu$ l of cell suspension into each well of a slide. Incubate 24 hours in a 37°C CO<sub>2</sub> incubator.
2. Wash the cells three times with PBS. Do not shake cells.
3. Add fix (3.7% formaldehyde) in PBS for 10 minutes at room temperature.
4. Wash the cells three times with PBS. Do not shake.
5. Permeabilized in 0.1% Triton-X100 in PBS for 3 minutes at room temperature.
6. Wash cells with PBS for 5 minutes at room temperature.
7. Cover cells with 400 $\mu$ l of 10% normal goat serum in PBS and incubate for 30 minutes at room temperature.
8. Wash the cells three times with PBS.
9. Incubate the cells with **2.5-5 $\mu$ g/ml anti-FAK, clone 4.47**, in PBS and incubate for 1 hour at room temperature.
10. Wash the cells three times, 5 minutes each with PBS.
11. Incubate the cells in the dark, with a 1:100 dilution of goat anti-mouse IgG fluorescein conjugated secondary antibody in PBS for 45 minutes at room temperature.
12. Wash the cells three times with PBS.
13. Mount and examine the cells under a fluorescent microscope.