

## Certificate of Analysis

**Anti-FAK, clone 2A7**  
(mouse monoclonal IgG<sub>1</sub>)  
Catalog # 05-182  
Lot # 0701050542

**Immunogen:** Mixture of affinity-purified tyrosine phosphoproteins from chick embryo fibroblasts expressing p125<sup>FAK</sup>. Clone 2A7.

**Specificity:** Recognizes and is specific for FAK.

**Species Cross-reactivity:** Human, rodent, and avian.

**Formulation:** 200 µg of protein G purified mouse IgG<sub>1</sub> in 200 µL PBS containing 0.035% sodium azide 30% glycerol. Liquid at -20°C.

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

**Note:** The FAK antibody shows different immunoaffinity for modified forms of FAK and alternate splicing transcripts of FAK.

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

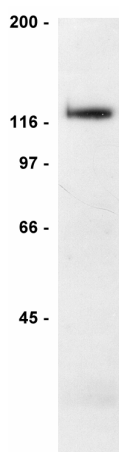
### Quality Control Testing

**Immunoprecipitation/Immunoblot:** 4 µg of this lot of antibody immunoprecipitated FAK from a mouse 3T3/A31 RIPA cell lysate (Catalog #12-305), as demonstrated by subsequent immunoblot analysis of the immunoprecipitate using a polyclonal FAK antibody (Catalog # 06-543).

### Additional Research Applications

**Western Immunoblot:** **Not recommended.** The antibody is ineffective for immunoblot analysis.

**Immunofluorescence:** Use at 10 µg/mL.



**Immunoprecipitation/Immunoblot Analysis:**  
Representative blot from a previous lot. 4 µg of monoclonal anti-FAK was used to immunoprecipitate FAK from 500 µg of a mouse 3T3/A31 cell RIPA lysate. The immunoprecipitate was resolved by electrophoresis, transferred to nitrocellulose and probed with polyclonal rabbit anti-FAK (Catalog # 06-543, 2 µg/mL). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates FAK.

### Application References:

1. Carlson, T., *et al.*, J. Biol. Chem. **276**: 26516-26525, 2001.
2. Fernandez-Rodriguez, *et al.*, J. Biol. Chem. **273**: 19321, 1998.
3. Kanner, S.B., *et al.*, Proc. Natl. Acad. Sci. USA **87**: 3328-3332, 1990.
4. Cobb, B.S., *et al.*, Mol. Cell. Biol. **14**: 147-155, 1994.
5. Schaller, M.D., *et al.*, Proc. Natl. Acad. Sci. USA **89**: 5192-5196, 1992.

### Immunoprecipitation/Immunoblot Protocol

1. Dilute the cell lysate (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL aprotinin, leupeptin, pepstatin; 1 mM Na<sub>3</sub>VO<sub>4</sub>; 1 mM NaF) before beginning the immunoprecipitation to roughly 1 µg/µL total cell protein in a microcentrifuge tube with PBS.
2. Add **4 µg of anti-FAK** to 500 µg-1 mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 µL (50 µL 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 µL 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or boiled for 5 minutes. Collect the beads using a microcentrifuge pulse.
9. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant.
10. Transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose once with PBS-0.05% Tween 20 for 20 minutes and twice with water.
11. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 20 minutes at room temperature with constant agitation.
12. Incubate the nitrocellulose with **2-4 µg/mL polyclonal anti-FAK (Catalog # 06-543)** diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
13. Wash the nitrocellulose twice with water.
14. 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 PBS-MLK for 1.5 hours at room temperature with agitation.
15. Wash the nitrocellulose with water twice.
16. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
17. Rinse the nitrocellulose in 4-5 changes of water.
18. Use detection method of choice (enhanced chemiluminescence was used).