

## Certificate of Analysis

**Anti-FAK, clone 2A7**  
(mouse monoclonal IgG<sub>1</sub>)  
Catalog # 05-182  
Lot # JBC1370010

**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 0.05% 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**

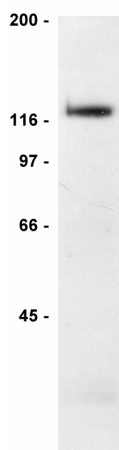
### Quality Control Testing

**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  $\mu\text{g}/\text{mL}$  aprotinin, leupeptin, pepstatin; 1 mM  $\text{Na}_3\text{VO}_4$ ; 1 mM NaF) before beginning the immunoprecipitation to roughly 1  $\mu\text{g}/\mu\text{L}$  total cell protein in a microcentrifuge tube with PBS.
2. Add **4  $\mu\text{g}$  of anti-FAK** to 500  $\mu\text{g}$ -1 mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100  $\mu\text{L}$  (50  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{g}/\text{mL}$  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).