

## Anti-Focal Adhesion Kinase (p125<sup>FAK</sup>)

(mouse monoclonal IgG<sub>1</sub>, clone 2A7)

Catalog # 05-182

Lot # 17763

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

**Source:** Mouse-mouse hybridoma (clone designation 2A7 [SP2/0 myeloma x AJ mouse splenocytes]) propagated as ascites.

**Specificity:** Recognizes and is specific for p125<sup>FAK</sup>.

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

**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.

**Formulation:** 200ng of protein G purified mouse IgG<sub>1</sub> in 277μl 0.1M Tris-glycine, pH 7.4, 0.15M NaCl with 0.05% sodium azide. Frozen solution.

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

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

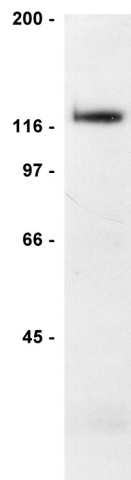
### Quality Control Testing

**Immunoprecipitation/Immunoblot:** 4μg of this immunoprecipitated FAK from a mouse 3T3 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 10μg/ml.



**Immunoprecipitation/Immunoblot Analysis**  
4μg of Monoclonal Anti-FAK was used to immunoprecipitate FAK from 500μg of a mouse 3T3 cell RIPA lysate. The immunoprecipitate was resolved by electrophoresis, transferred to nitrocellulose and probed with polyclonal rabbit anti-FAK (Catalog # 06-543, 2μg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates p125<sup>FAK</sup>.

### Application References:

Kanner, S.B., *et al.*, Proc. Natl. Acad. Sci. USA **87**: 3328-3332, 1990.  
Cobb, B.S., *et al.*, Mol. Cell. Biol. **14**: 147-155, 1994.  
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: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 $\mu$ g/ml aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 1mM NaF) 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 a-FAK** 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 of washed Protein G agarose bead slurry (50 $\mu$ l packed beads).
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.
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 Tween 20 for 20 minutes and twice with water.
11. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
12. Incubate the nitrocellulose with **2-4 $\mu$ g/ml polyclonal a-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 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).