



cell signaling solutions

Certificate of Analysis

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Anti-FAK, clone 2A7

(mouse monoclonal IgG₁)

Catalog # 05-182

Lot # 23166

Immunogen: Mixture of affinity-purified tyrosine phosphoproteins from chick embryo fibroblasts expressing p125^{FAK}. Clone 2A7.

Specificity: Recognizes and is specific for FAK.

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: 200µg of protein G purified mouse IgG₁ in 352µl 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

Note: The FAK antibody shows different immunoreactivity 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 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 10µg/ml.



Immunoprecipitation/Immunoblot Analysis:

Representative blot from a previous lot. 4µg of monoclonal anti-FAK was used to immunoprecipitate FAK from 500µg 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, 2µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates FAK.

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 μ g/ml aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) before beginning the immunoprecipitation to roughly 1 μ g/ μ l total cell protein in a microcentrifuge tube with PBS.
2. Add **4 μ g of anti-FAK** to 500 μ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 μ l (50 μ 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 50 μ 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-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 μ g/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).