



cell signaling solutions

Certificate of Analysis

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Anti-FAK, BC3 (rabbit polyclonal IgG) Catalog # 06-446 Lot # 28848

Immunogen: TrpE-Fak fusion protein containing amino acids 651-1028 of chicken FAK.

Specificity: Recognizes and is specific for FAK, BC3, Mr 125kDa.

Species Cross-reactivity: Human, rodent and avian.

Formulation: 150µg of protein G purified rabbit IgG in 150µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, with 0.05% sodium azide. Frozen solution.

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 the product, centrifuge the original vial prior after thawing and prior to removing the cap.

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

Quality Control Testing

Immunoprecipitation: 4µg of this lot of antibody immunoprecipitated FAK from a 3T3/A31 cell lysate (Catalog # 12-305), and previously from a Src-transformed chicken cell line lysate (500µg-1mg). Confirmed by subsequent immunoblot analysis of the immunoprecipitate using 1-2µg/ml of anti-FAK, clone 4.47 (Catalog # 05-537).

Additional Research Applications

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

Immunofluorescence: 10µg/ml of this antibody has been shown by an independent laboratory to detect FAK in chicken embryo fibroblasts.

Application References:

1. Schaller, M.D., *et al.*, Proc. Natl. Acad. Sci. USA 89: 5192-5196, 1992.
2. Cobb, B.S., *et al.*, Mol. Cell Biol. 14: 147-155, 1994.
3. Kanner, S.B., *et al.*, Proc. Natl. Acad. Sci. USA 87: 3328-3332, 1990.

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 EDTA; 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 A agarose bead slurry (Catalog # 16-125).
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 μ l 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes. Collect the beads by 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 (PBS-MLK) for 20 minutes at room temperature with constant agitation.
12. Incubate the nitrocellulose with **1-2 μ g/ml of anti-FAK, clone 4.47 (Catalog # 05-537)**, 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-mouse HRP conjugated IgG, Catalog # 12-349, 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).