

Anti-FAK
(rabbit polyclonal IgG)
Catalog # 06-446
Lot # 18880

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

Specificity: Recognizes and is specific for FAK, 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 and Research Applications

Western Immunoblot: Not recommended.

Immunoprecipitation: 4µg of this lot immunoprecipitated Fak from a 3T3 RIPA cell lysate, and previously from a Src-transformed chicken cell line lysate (500µg-1mg). Confirmed by subsequent immunoblot analysis using 1-4µg/ml of a polyclonal FAK antibody (Catalog # 06-543).

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 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 μg of anti-FAK** to $500\mu\text{g}$ -1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding $100\mu\text{l}$ of washed Protein A agarose bead slurry ($50\mu\text{l}$ 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 $14,000 \times g$), 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 $50\mu\text{l}$ 2X Laemmli sample buffer. The agarose beads can either be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes.
8. Collect the beads by a microcentrifuge pulse.
9. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a sample of the supernatant and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
10. Block the blotted nitrocellulose in PBS-0.05% Tween for 20 minutes at 20 - 25°C with constant agitation.
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 **1-4 $\mu\text{g}/\text{ml}$ of 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 IgG** linked to horseradish peroxidase, 1:3000 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).