

Anti-Focal Adhesion Kinase (p125^{Fak})
(mouse monoclonal IgG₁)

Catalog #05-182

Lot # 16028

Immunogen: Mixture of affinity-purified tyrosine phosphoproteins from chick embryo fibroblasts expressing p125^{Fak}.

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

Antibody Class: IgG₁.

Purification Method: Protein G-Sepharose Chromatography.

Specificity: Recognizes and is specific for p125^{Fak}.

Species Cross-reactivity: Species cross-reactivity includes human, rodent, and avian.

Note: The Fak antibody shows different immunoaffinity for modified forms of Fak and alternate splicing transcripts of Fak.

Quantity and Formulation: 200ng protein G purified IgG₁ in 217ml 0.1M Tris-glycine, pH 7.0.

Physical Form: Frozen solution.

Storage and Stability: 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.

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.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing and Research Applications

Immunoprecipitation (IP): 4 μ g of this lot was used with Protein G agarose to immunoprecipitate Fak from a RIPA lysate of mouse 3T3 cells (Catalog #12-305), as demonstrated by immunoblot analysis of the immunoprecipitate using an anti-Fak polyclonal antibody (Catalog #06-543).

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

Immunofluorescence: Use at 10 μ g/ml.

Immunoprecipitation 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 a-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 of washed Protein G agarose bead slurry (50 μ 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 μ l 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or boiled for 5 minutes. The beads are collected by a microcentrifuge pulse and SDS-PAGE and subsequent immunoblot analysis can be performed on a sample of the supernatant.