

Anti-FAK

Polyclonal Antibody

Cat. # 06-543

Lot # DAM1522865

pack size: 200 µg

Store at -20°C

FOR RESEARCH USE ONLY



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IP	H, Ht, M, R	IgG	N/A	Rb	125 kDa	NM_005607 / NM_153831

Background

Focal adhesion kinase (FAK) is a non receptor protein tyrosine kinase discovered as a substrate for Src and as a key element of integrin signaling. FAK plays a central role in cell spreading, differentiation, migration, cell death and acceleration of the G1 to S phase transition of the cell cycle. FAK regulation includes phosphorylation at multiple tyrosine and serine residues. Phosphorylation of tyrosine generally is associated with positive regulation and growth promotion, however, dephosphorylation at these sites occurs as cells enter mitosis (M-Phase of the cell cycle). In contrast, serine phosphorylation either remains high or is increased as cells enter mitosis and may play a role in focal adhesion disassembly. FAK and its phosphorylation states have been implicated in cancer metastasis and tumor cell survival and adhesion-independent growth. Additionally, recent evidence indicates that elevation of FAK activity in human carcinoma cells is associated with increased invasive potential. A central role in tumor formation and progression suggests that FAK is an attractive target for therapeutic intervention.

Presentation

Purified rabbit polyclonal IgG in buffer containing storage buffer (0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, 0.05% sodium azide). Store at -20°C.

Concentration

1 mg/mL

Specificity

Recognizes and is specific for human p125^{FAK}, does not cross-react with Pyk2.

Species Cross-reactivity

Human, mouse, rat and hamster not avian.

Immunogen

pGEX-derived fusion protein containing residues 748-1053 of human FAK

Molecular Weight

125 kDa

Method of Purification

Protein A Purified

Storage and Handling

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.

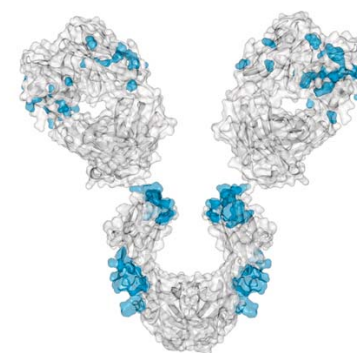
Control

Included Positive Antigen Control: Catalog # 12-305, 3T3/A31 cell lysate. Add 2.5 µL of 2-mercaptoethanol/100 µL of lysate and boil for 5 minutes to reduce the preparation. Load 20 µg of reduced lysate per lane for minigels.

Quality Control Testing

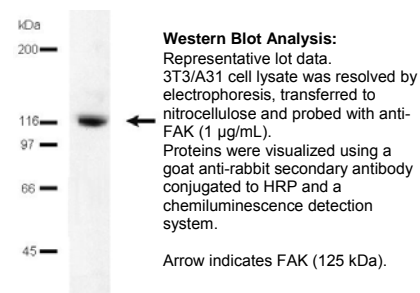
Evaluated by western blot on RIPA lysates from murine 3T3/A31 or human A431 RIPA cell lysate.

Western Blot Analysis: 0.5-2 µg/mL of this antibody detected FAK in RIPA lysates from murine 3T3/A31 and previously in human A431 RIPA cell lysate.



References

1. Tsopanoglou, N. E. *et al.* (2004). *Thromb. Haemost.* 92:846-857.
2. Ohtaki, T. *et al.* (2001). *Nature.* 411:613-617
3. Zabe, M. & Dean, W. L. (2001). *J. Biol. Chem.* 276:14704-9.
4. Gervais, F. G. *et al.* (1998). *J. Biol. Chem.* 273:17102-8.
5. Saxena, S. P. *et al.* (1997). *J. Clin. Invest.* 99:602-607.



APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence
IH Immunohistochemistry (Tissue)

SPECIES LEGEND: H Human Ht Hamster M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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Additional Research Applications

Immunocytochemistry: 10 µg/mL of a previous lot showed positive immunostaining for Fak in Ref 52 cells fixed with 3.7% formaldehyde.

Immunoprecipitation: 4 µg of a previous lot immunoprecipitated FAK from 500 µg of murine 3T3/A31 RIPA lysate.

PROTOCOL

Western Blot

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na₃VO₄; 1 mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in PBS-0.05% Tween 20 for 20 minutes at room temperature with constant agitation.
3. 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.
4. Incubate the nitrocellulose with 0.5-2 µg/mL of anti-FAK, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
5. Wash the nitrocellulose twice with water.
6. 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.
7. Wash the nitrocellulose with water twice.
8. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
9. Rinse the nitrocellulose in 4-5 changes of water.
10. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation

1. Add 4 µg of anti-FAK and 60 µL (30 µL packed beads) of washed Protein A agarose bead slurry (Catalog # 16-125) to 500 µL of PBS in a microcentrifuge tube.
2. Gently rock the reaction mixture at 4°C for 1 hour.
3. 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.
4. Dilute the cell lysate to roughly 1 µg/µL total cell protein with PBS.
5. Add 500 µg-1 mg cell lysate to the reaction mixture.
6. Gently rock the reaction mixture at 4°C for 1 hour.
7. 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.
8. Resuspend the agarose beads in 70 µL 2X Laemmli sample buffer.
9. Store the beads frozen for future analysis or boil the beads for 5 minutes.
10. Collect the beads after boiling using a microcentrifuge pulse.
11. Perform SDS-PAGE and western blot analysis on a sample of the supernatant fraction.

Immunocytochemistry

1. Plate approximately 200 µL of cell suspension into each well of a slide. Incubate 24 hours in a 37°C CO₂ incubator.
2. Wash the cells three times with PBS.
3. Add fix (3.7% of formaldehyde) in PBS for 10 minutes at room temperature.
4. Wash the cells three times with PBS.
5. Permeabilize with 0.1% Triton-X100/PBS for 3 min at room temperature.
6. Wash the cells three times with PBS.
7. Cover cells with 400 µL of 1% BSA in PBS and incubate for 30 minutes at room temperature.
8. Wash the cells with three times with PBS.
9. Incubate the cells with 10 µg/mL anti-FAK in 1% BSA in PBS and incubate for 1 hour at room temperature.
10. Wash the cells three times with PBS.
11. Incubate the cells, in the dark with a 1:100 dilution of goat anti-rabbit IgG fluorescein conjugated secondary antibody in 1% BSA in PBS for 45 minutes at room temperature (keep in dark).
12. Wash the cells three times with PBS.
13. Mount and examine the cells under a fluorescent microscope.

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05-537	■ Anti-FAK, clone 4.47
16-173	■ Anti-FAK, clone 4.47, agarose conjugate
16-233	■ Anti-FAK, clone 4.47, Alexa Fluor® 488 conjugate
16-234	■ Anti-FAK, clone 4.47, Alexa Fluor® 555 conjugate
04-009	■ anti-FAK, clone HL563
04-591	■ Anti-FAK, Rabbit Monoclonal, clone EP695Y
MAB2156	■ Anti-Focal Adhesion Kinase, clone BLAb2H7
AB1605	■ Anti-Focal Adhesion Kinase, C-terminus
MAB1144	■ Anti-Focal Adhesion Kinase, phospho-specific (Tyr397)
07-825	■ Anti-phospho-FAK (Ser722)
07-826	■ Anti-phospho-FAK (Ser732)
07-827	■ Anti-phospho-FAK (Ser843)
07-828	■ Anti-phospho-FAK (Ser910)
07-829	■ Anti-phospho-FAK (Ser407)
07-830	■ Anti-phospho-FAK (Ser576)
07-157	■ Anti-phospho-FAK (Ser576)
07-831	■ Anti-phospho-FAK (Ser577)
07-832	■ Anti-phospho-FAK (Ser861)
14-720	■ FAK, active
17-177	■ CAKPAK, (Anti-FAK, Pyk 2, ILK), Miniature Set
17-480	■ Phospho-FAK (Tyr397) STAR ELISA Kit
17-479	■ STAR FAK ELISA Kit
M-003164	■ FAK (PTK2) SMARTpool® siRNA reagent
60-018	■ FAK (PTK2) siRNA/siAb™ Assay Kit
62-174	■ siRNA plasmid, pKD-FAK-v1
62-175	■ siRNA plasmid, pKD-FAK-v4
12-348	■ Goat Anti-Rabbit IgG
17-500	■ Catch and Release Reversible Immunoprecipitation System
16-266	■ Protein G Agarose Fast Flow 10mL
16-125	■ Protein A-Agarose 10ML

RELATED PRODUCTS (non-specific)

cat #	description
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IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

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