Rabbit (polyclonal) Anti-FAK [pY^{407}] Phosphospecific Antibody, Unconjugated

Catalog no. 44650G

(See product label for lot information)

Clone/PAD: pAb
Isotype: Rb IgG
Gene ID: PTK2
Qty: 10 mini-blot size
Volume: 100 µL

Formulation
Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.

Validation
See www.invitrogen.com/antibodies for protocols
Validated for use in WB, ICC, IHC

Reactivity
Human FAK. Mouse, rat, chicken (100% homologous) and frog (92% homologous) FAK have not been tested, but are expected to react.

Immunogen
Synthetic phosphopeptide from human FAK containing tyrosine 407. The sequence is conserved in mouse, rat and chicken.

Sequence Identity
Human FAK.

Sequence Homology
Mouse, rat, chicken (100% homologous) and frog (92% homologous) FAK have not been tested, but are expected to react.

Storage
Store at −20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at −20°C. For short-term storage (up to one week), 2-8°C is sufficient.

Expiration Date
Expires one year from date of receipt when stored as instructed.

Background
Focal Adhesion Kinase (FAK) is a 125 kDa non-receptor protein tyrosine kinase that was discovered as a substrate for Src, and is 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. Phosphorylation of tyrosine 407 is activated by TGFβ and Epithelial Mesenchyme Transition (EMT). Unlike other sites on FAK, tyrosine 407 is EGFR independent.

Applications
The antibody has been used in Western blotting, immunocytochemistry and immunohistochemistry.

Application Use
For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The optimal antibody concentration should be determined empirically for each specific application.

Test Material
Chicken embryo fibroblast (CEF) cells expressing FAK protein and plated on fibronectin.

Purification
Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using (1) a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated FAK and (2) a generic tyrosine phosphorylated peptide to remove antibody that is reactive with phosphotyrosine, irrespective of the sequence. The final product is generated by affinity chromatography using a FAK-derived peptide that is phosphorylated at tyrosine 407.

Preservative
0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)

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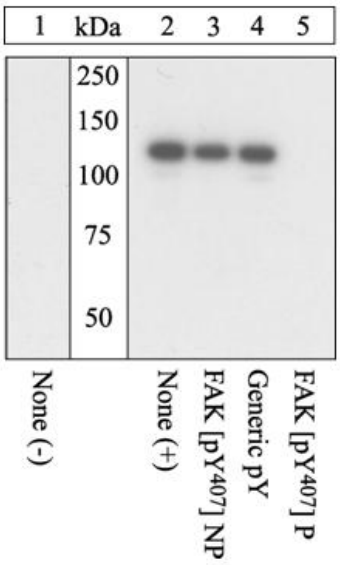

Peptide Competition

Extracts of primary chick embryo fibroblasts plated on fibronectin (1) and expressing human FAK (2-5) were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 5% BSA-TBST buffer for one hour at room temperature, then incubated with the FAK [pY^{407}] antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 2), the non-phosphopeptide corresponding to the phosphopeptide immunogen (3), a generic phosphotyrosine-containing peptide (4), or the phosphopeptide immunogen (5). After washing, the membrane was incubated with goat F(ab')$_2$ anti-rabbit IgG-HRP conjugate (Cat. # ALI4404), and signals were detected using the Pierce SuperSignal™ method.The data show that only the phosphopeptide corresponding to FAK [pY^{407}] blocks the antibody signal, demonstrating the specificity of the antibody.

References

1. Lu, H., et al. (2006) The microtubule binding drug laulimalide inhibits VEGF-induced human endothelial cell migration, and is synergistic when combined with Taxotere (docetaxel). Mol. Pharmacol. [Epub ahead of print] (cites the use of cat. # 44-614G, 44-624G, 44-626G, 44-650G and 44-652G).
2. Eliceiri, B.P., et al. (2002) Src-mediated coupling of focal adhesion kinase to integrin alpha(v)beta5 in vascular endothelial growth factor signaling. J. Cell. Biol. 157(1):149-160 (cites the use of cat. # 44-614G, 44-616 (discontinued), 44-624G, 44-626G, 44-650G and 44-652G).
3. Lim, Y., et al. (2002) Trichostatin A-induced detransformation correlates with decreased focal adhesion kinase phosphorylation at tyrosine 861 in ras-transformed fibroblasts. J. Biol. Chem. 277(15):12735-12740 (cites the use of cat. # 44-614G, 44-616 (discontinued), 44-624G, 44-626G, 44-650G and 44-652G).
4. Nakamura, K., et al. (2001) Different modes and qualities of tyrosine phosphorylation of Fak and Pyk2 during epithelial-mesenchymal transdifferentiation and cell migration: analysis of specific phosphorylation events using site-directed antibodies. Oncogene 20(21):2626-2635 (cites the use of cat. # 44-614G, 44-616 (discontinued), 44-618G, 44-620G, 44-624G, 44-626G, 44-632G, 44-634G, 44-650G and 44-656G).
5. Datta, A., et al. (2001) Transformation of chicken embryo fibroblasts by v-src uncouples β1 integrin-mediated outside-in but not inside-out signaling. Mol. Cell. Biol. 21(21):7295-7306 (cites the use of cat. # 44-614G, 44-616 (discontinued), 44-624G, 44-626G and 44-650G).
6. Vial, D., et al. (2000) The NH2 terminal region of FAK reconstitutes high affinity IgE receptor induced secretion in mast cells. J. Biol. Chem. 275(36):28269-28275 (cites the use of cat. # 44-614G, 44-616 (discontinued), 44-624G, 44-626G, 44-650G and 44-652G).

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