

Product Information

Anti-phospho-Integrin $\beta 3$ (pTyr⁷⁷³)

produced in rabbit, affinity isolated antibody

Catalog Number **I7408**

Product Description

Anti-phospho-Integrin $\beta 3$ (pTyr⁷⁷³) is produced in rabbit using a synthetic phosphorylated peptide derived from the region of human integrin $\beta 3$ receptor that contains tyrosine 773 (based on Swiss Protein database, accession number P05106). The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated Integrin $\beta 3$ peptide.

The antibody detects human Integrin $\beta 3$. Other species, which have not been tested, include: mouse and rat (100% homology), chicken (94%), and frog (88%) Integrin $\beta 3$. These species may cross-react. The antibody has been used in immunoblotting applications.

Integrin $\beta 3$, also known as CD61, is a 130 kDa transmembrane glycoprotein that binds noncovalently in complexes with integrin (subunits α_{IIb} , α_v) to form the functional receptor that binds to specific extracellular matrix proteins, e.g., fibronectin, vitronectin, etc.. Integrin receptors are involved in the regulation of a variety of important biological functions, including embryonic development, wound repair, hemostasis, and prevention of programmed cell death. They are also implicated in abnormal pathological states such as tumor directed angiogenesis, tumor cell growth, and metastasis. These heterodimeric receptors bridge the cytoplasmic actin cytoskeleton with proteins present in the extracellular matrix and/or on adjacent cells. The clustering of integrin receptors on the cell surface and their binding to the extracellular matrix leads to the formation of focal contacts and the activation of various signal transduction pathways.

Phosphorylation of tyrosine 773 on Integrin $\beta 3$ activates the receptor and promotes cell adhesion. Tyrosine 773 is commonly referred to as Tyrosine 747, the corresponding site in the chicken Integrin $\beta 3$ protein.

Reagent

Supplied in Dulbecco's PBS with 50% glycerol and 1.0 mg/mL BSA.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at $-70\text{ }^{\circ}\text{C}$. Upon initial thawing, freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 blots.

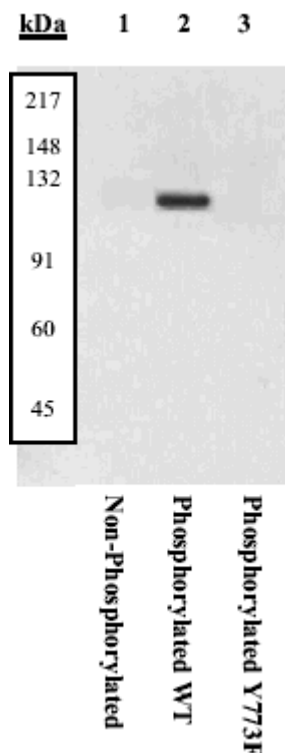
Immunoblotting: a recommended working concentration of 0.1-1.0 $\mu\text{g/mL}$ is determined using K562 cells transfected with human Integrin α_v and wild-type (WT) or mutant (Y773F) human Integrin $\beta 3$, and analyzed by immunoprecipitation with 1A2 (human Integrin $\beta 3$) monoclonal antibody.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Western Blot

1. K562 cells transfected with human Integrin α_v and wild-type (WT) or mutant (Tyr^{773F}) human Integrin $\beta 3$ were analyzed by immunoprecipitating the Integrin $\beta 3$ protein with 1A2 (human Integrin $\beta 3$) mAb, and resolved by SDS-PAGE on a 10% Tris-glycine gel.
2. The proteins then were transferred to PVDF and, after blocking with a 5% BSA-TBST buffer overnight at $4\text{ }^{\circ}\text{C}$, preincubated with:
Lane 1 non-phosphorylated Integrin $\beta 3$
Lane 2 phosphorylated WT Integrin $\beta 3$
Lane 3 phosphorylated Tyr^{773F} mutant Integrin $\beta 3$

3. All lanes were incubated with 0.50 µg/mL Integrin β3 [pTyr⁷⁷³] antibody .
4. After washing, membranes were incubated with goat F(ab')₂ anti-rabbit IgG alkaline phosphatase and bands were detected.



The data show detection of Integrin β3 receptor phosphorylation with the wild-type but not the mutant recombinant protein, thereby demonstrating the specificity of the Integrin β3 [pTyr⁷⁷³] antibody.

References

1. Maile, L.A., et al. Structural analysis of the role of the β3 subunit of the α V β3 integrin in IGF-I signaling. *J. Cell Sci.*, **114**, 1417-1425 (2001).
2. Sanjay, A., et al., Cbl associates with Pyk2 and Src to regulate Src kinase activity, α(v)β(3) integrin-mediated signaling, cell adhesion, and osteoclast motility. *J. Cell Biol.* **152**, 181-195 (2001).
3. Patil, S., et al. Identification of a talin-binding site in the integrin β(3) subunit distinct from the NPLY regulatory motif of post-ligand binding functions. The talin n-terminal head domain interacts with the membrane-proximal region of the β(3) cytoplasmic tail. *J. Biol. Chem.* **274**, 28575-28583 (1999).
4. Schlaepfer, D.D. and T. Hunter, Integrin signalling and tyrosine phosphorylation: just the FAKs? *Trends Cell Biol.* **8**, 151-157 (1998).
5. Blystone, S.D., et al., Requirement of integrin β3 tyrosine 747 for β3 tyrosine phosphorylation and regulation of αvβ3 avidity. *J. Biol. Chem.* **272**, 28757-28761 (1997).
6. Richardson, A. and J.T. Parsons Signal transduction through integrins: a central role for focal adhesion kinase? *Bioessays*, **17**, 229-236 (1995).

FF,PHC 06/11-1