Rabbit (polyclonal) Anti-Integrin β1 Receptor [pTpT$^{788/789}$] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number/Size:	44-872G (10 mini-blot size)
Lot Number:	See product label
Volume:	100 µL
Form of Antibody:	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.
Preservative:	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated integrin β1 receptor protein. The final product is generated by affinity chromatography using an integrin receptor-derived peptide that is phosphorylated at threonine 788 and threonine 789.
Immunogen:	The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human integrin β1 receptor that contains threonines 788 and 789 (based on Swiss Protein database, accession number P05558). The sequence is conserved in human, mouse, rat and chicken.
Target Summary:	Integrin β1, also known as CD29, is a 130 kDa transmembrane glycoprotein that forms non-covalent complexes with various integrin α subunits (including α1, α2, α3, α4, α5, and α6, also known as CD49a, CD49b, CD49c, CD49d, CD49e, and CD49f, respectively) to form the functional receptors that bind to specific extracellular matrix proteins. 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 a cell's surface leads to the formation of focal contacts. Interactions between integrin receptors and the extracellular matrix lead to activation of signal transduction pathways and regulation of gene expression. Phosphorylation of threonines 788 and 789 on the integrin β1 receptor may play a key role in cell-cycle dependent regulation.
Reactivity:	Human integrin β1 receptor. Mouse, rat and chicken (100% homologous) integrin β1 receptor have not been tested, but are expected to react.
Applications:	The antibody has been used in Western blotting.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The optimal antibody concentration should be determined emperically for each specific application.
Positive Control Used:	HeLa cells undergoing mitosis
Storage:	Store at –20ºC. 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 two weeks), 2-8°C is sufficient.
Expiration Date:	Expires one year from date of receipt when stored as instructed.

This product is for research use only. Not for use in diagnostic procedures.

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Related Products:

FAK [pY^{397}], Cat. # 44-624G
Src [pY^{418}], Cat. # 44-660G
ERK1&2 [pTpY$^{185/187}$], Cat. # 44-680G
Integrinβ1[pS^{785}], Cat. # 44-870G
Integrinβ3[pY^{773}], Cat. # 44-876G
Integrinβ3[pY^{785}], Cat. # 44-878

References:

Hammar, E., et al. (2004) Extracellular matrix protects pancreatic ß-cells against apoptosis: role of short- and long-term signaling pathways. Diabetes 53:2034-2041 (cites the use of cat. # 44-624G and AHO0502).

Schliess, F., et al. (2004) Involvement of integrins and Src in insulin signaling toward autophagic proteolysis in rat liver. J. Biol. Chem. 279(20):21294-21301 (cites the use of cat. # 44-624G, 44-660G, 44-802G and 44-655G).

Liu, B.P. and K. Burridge (2000) Vav2 activates Rac1, Cdc42, and RhoA downstream from growth factor receptors but not beta1 integrins. Mol. Cell. Biol. 20(19):7160-7169.

Arthur, W.T., et al. (2000) Integrin engagement suppresses RhoA activity via a c-Src-dependent mechanism. Curr. Biol. 10(12):719-722.

Blystone, S.D., et al. (1999) A molecular mechanism of integrin crosstalk: alphavbeta3 suppression of calcium/calmodulin-dependent protein kinase II regulates alpha5beta1 function. J. Cell. Biol. 145(4):889-897.

Schlaepfer, D.D., et al. (1999) Signaling through focal adhesion kinase. Prog. Biophys. Mol. Biol. 71(3-4):435-478.

Wennerberg, K., et al. (1998) Mutational analysis of the potential phosphorylation sites in the cytoplasmic domain of integrin β1a: Requirement for threonines 788-789 in receptor activation. J. Cell. Sci. 111 (Pt 8):1117-1126.

Schlaepfer, D.D. and T. Hunter (1998) Integrin signalling and tyrosine phosphorylation: just the FAKs? Trends Cell Biol. 8(4):151-157.

Richardson, A. and J.T. Parsons (1995) Signal transduction through integrins: a central role for focal adhesion kinase? Bioessays 17(3):229-236.

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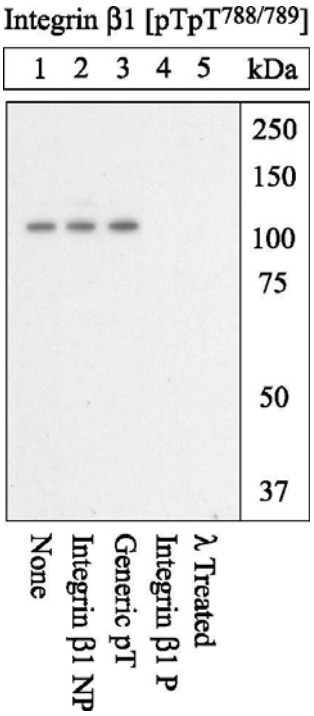

Peptide Competition and Phosphatase Stripping

Extracts of serum-starved mitotic HeLa cells generated by treatment with 100 ng/mL taxol for 16 hours were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was either untreated (1-4) or treated with lambda (λ) phosphatase (5), blocked with a 5% BSA-TBST buffer for one hour at room temperature, then incubated with the Integrinβ1 [pTpT$^{788/789}$] antibody for two hours at room temperature in a 3% BSA-TBST buffer, following its prior incubation with: no peptide (1,5), the non-phosphorylated peptide corresponding to the phosphopeptide immunogen (2), a generic phosphothreonine-containing peptide (3), or the phosphopeptide immunogen (4). 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 Integrinβ1 [pTpT$^{788/789}$] blocks the antibody signal, demonstrating the specificity of the antibody. The data also show that phosphatase stripping eliminates the signal, further verifying that the antibody is phospho-specific.

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Western Blotting Procedure

1. Lyse approximately 10^7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Invitrogen catalog number FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of nitrocellulose membrane slightly larger than the gel.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4°C or for one hour at room temperature.
12. Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for two hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (catalog number ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (catalog number ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma catalog number P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
5 gm Ig-free BSA
0.1 mL Tween 20

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Peptide Competition Experiment

Invitrogen's Phosphorylation Site Specific Antibodies (PSSAs) have been developed to enable the specific and sensitive detection of phosphorylation of particular amino acid residues in target proteins, while circumventing the need for protein purification, phosphopeptide mapping or handling radioactivity. The specificity of a PSSA in each experimental system can be confirmed through peptide competition. In this technique, aliquots of antibody are pre-incubated with peptide containing the sequence of the phosphopeptide immunogen used to raise the PSSA and the corresponding non-phosphopeptide. Following preincubation with the peptide, each antibody preparation is then used as a probe in antibody-based detection methods, such as Western blotting, immunocytochemistry, flow cytometry, or ELISA. With a PSSA specific for the phosphorylated target protein, pre-incubation with an excess of peptide containing the sequence of the phosphopeptide immunogen will block all antigen binding sites, while pre-incubation with the corresponding non-phosphopeptide will not affect the antibody-antigen interaction.

Invitrogen has developed a line of control peptides specifically for use in peptide competition experiments with our PSSAs. These peptides, available as separate Invitrogen catalog items, are provided in pairs which contain the sequences of the phosphopeptide immunogen and the corresponding non-phosphopeptide.

In performing the Peptide Competition Experiment, it is important to note that the optimal dilutions of both antibody and peptide should be determined empirically for each specific application. The optimal dilution of antibody in these procedures is below saturating, as determined by previous experiments in your system. If an optimal antibody dilution has not been determined in your system, please refer to the Suggested Working Dilution on the antibody Product Analysis Sheet for guidance on an appropriate starting dilution. The optimal dilution of peptide used in these procedures will depend on the overall affinity or avidity of the antibody, as well as the quantity of the target antigen. A 50-150 fold molar excess of peptide to antibody is found to be effective for most peptide competition experiments.

In the example presented below, the PSSA is used at a dilution of 1:1000 and the peptides are used at a concentration of 333 nM. The total volume of the phosphopeptide and non-phosphopeptide-pre-incubated antibody preparations is 2 mL, sufficient for probing Western blot strips, as well as for use in other antibody-based detection methods. Under these conditions, the molar excess of peptide to antibody is ≥50.

Procedure:

1. Prepare three *identical test samples*, such as identical PVDF or nitrocellulose strips to which the protein of interest has been transferred. The test samples should be blocked using a blocking buffer, such as Tris buffered saline supplemented with 0.1% Tween 20, and either 5% BSA or 5% non-fat dried milk.
2. Prepare 6.5 mL of *working antibody stock solution* (1:1000 in this example) by adding 6.5 µL of antibody stock solution to 6.5 mL of buffer containing blocking protein, such as TBS supplemented with 0.1% Tween 20, and either 3% BSA or 3% non-fat dried milk.
3. Apportion the unused PSSA into working aliquots and store at –20°C for future use (the stock PSSA contains 50% glycerol and will not freeze at this temperature).
4. Allow the *lyophilized control peptides* to reach room temperature, ideally under desiccation.
5. Reconstitute each of the control peptides (supplied at 0.1 mg/vial) to a concentration of 66.7 µM with nanopure water. For a peptide with a molecular mass of 1500 (stated on the peptide Product Analysis Sheet), reconstitution with 1 mL water yields a solution with a concentration of 66.7 µM.
6. Apportion the unused reconstituted peptide solutions into working aliquots and store at -20°C for future use.
7. Label 3 test tubes as follows:
   - tube 1: water only, no peptide control
   - tube 2: phosphopeptide
   - tube 3: non-phosphopeptide
8. Into each tube, pipette the following components
   - tube 1: 2 mL diluted PSSA solution plus 10 µL nanopure water
   - tube 2: 2 mL diluted PSSA solution plus 10 µL phosphopeptide
   - tube 3: 2 mL diluted PSSA solution plus 10 µL non-phosphopeptide
9. Incubate the three tubes for 30 minutes at room temperature with gentle rocking.
10. At the end of the incubation step, transfer the contents of each of the three tubes to three clean reaction vessels. Into each reaction vessel, transfer an identical test sample.

For Western blotting strips:

- Incubate the strips with the pre-incubated antibody preparations for 1 hour at room temperature or overnight at 4°C.
- Wash each strip four times, five minutes each, to remove unbound antibody.
- Transfer each strip to a new solution containing a labeled secondary antibody [e.g., goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404)].
- Remove unbound secondary antibody by thorough washing, and develop the signal using your chemiluminescent reagents and instrumentation.

The signal obtained with antibody incubated with the "Water Only, No Peptide Control" (Tube 1), represents the maximum signal in the assay. This signal should be eliminated by pre-incubation with the "Phosphopeptide" (Tube 2), while pre-incubation with the "Non-Phosphopeptide" (Tube 3) should not impact the signal. If the "Phosphopeptide" only partially eliminates the signal, repeat the procedure using twice the volume of water or peptide solutions listed in Step 8. If partial competition is seen following pre-incubation with the "Non-Phosphopeptide", repeat the procedure using half the volumes of water or peptide solutions listed in Step 8.

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