Rabbit (polyclonal) Anti-SHP2 [pS^{576}] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44-557G (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 SHP2. The final product is generated by affinity chromatography using a SHP2-derived peptide that is phosphorylated at serine 576.
Immunogen:	The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human SHP2 that contains serine 576. The sequence is conserved in mouse, rat and chicken.
Target Summary:	SHP2 (SH2-domain containing tyrosine phosphatase 2, also known as SHPTP2) is a ~70 kDa tyrosine phosphatase that plays an important role in cell growth and transformation and in mediating integrin, Src and receptor tyrosine kinase (RTK) signaling. SHP2 undergoes tyrosine phosphorylation on several sites including tyrosines 542 and 580 upon binding to platelet-derived growth factor receptor beta (PDGFRβ), in response to PDGF stimulation. Serines 576 and 591 are phosphorylated by protein kinase C isoforms alpha, beta 1, beta 2 and eta, and in response to phorbol ester.
Reactivity:	Human SHP2. Mouse and rat SHP2 (100% homologous) have not been tested, but are expected to react.
Applications:	The antibody has been used in Western blotting. Other applications have not been tested at Invitrogen.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1,000 starting dilution. The optimal antibody concentration should be determined empirically for each specific application.
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 shipment or 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.
Positive Control Used:	Hek293 treated with PMA.

Related Products:

Antibodies:

SHP2 [pY^{542}], Cat. # 44-554G	Src [pY^{418}], Cat. # 44-660G
SHP2 [pY^{580}], Cat. # 44-558G	ERK1&2 [pTpY$^{185/187}$], Cat. # 44-680G
Akt/PKB [pT^{308}], Cat. # 44-602G	IR/IGF1R [pYpYpY$^{1158/1162/1163}$], Cat. # 44-806G
Akt/PKB [pS^{473}] monoclonal, Cat. # 44-621G	Akt/PKB, Cat. # 44-609G
	Grb2, Cat. # AHO0512

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

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References:

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Araki, T., et al. (2003) Tyrosyl phosphorylation of Shp2 is required for normal ERK activation in response to some, but not all, growth factors. J. Biol. Chem. 278(43):41677-41684.

Chen, Y., et al. (2003) Identification of Shp-2 as a Stat5A phosphatase. J. Biol. Chem. 278(19): 16520-16527.

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Strack, V., et al. (2002) The Protein-tyrosine-phosphatase SHP2 is phosphorylated on serine residues 576 and 591 by protein kinase C isoforms alpha, beta 1, beta 2, and eta. Biochemistry 41(2):603-608.

Vadlamudi, R.K., et al. (2002) Differential regulation of components of the focal adhesion complex by heregulin: role of phosphatase SHP-2. J. Cell. Physiol. 190(2):189-199.

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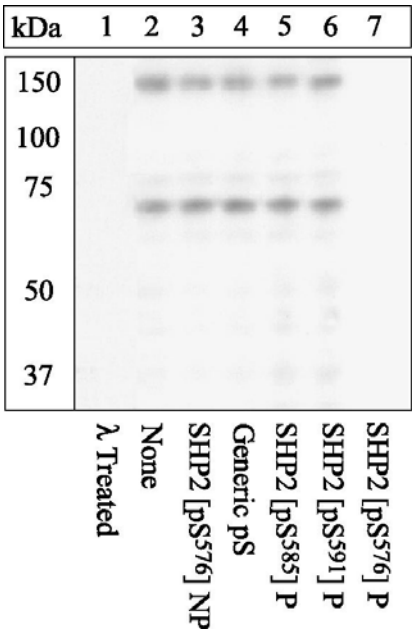
Lu, W., et al. (2001) Site-specific incorporation of a phosphotyrosine mimetic reveals a role for tyrosine phosphorylation of SHP-2 in cell signaling. Mol. Cell. 8(4):759-769.

Bennett, A.M., et al. (1994) Protein-tyrosine-phosphatase SHPTP2 couples platelet-derived growth factor receptor beta to Ras. Proc. Nat'l. Acad. Sci. USA 91(15):7335-7339.



Antibody-Peptide Competition and Phosphatase Treatment

Extracts of Hek293 cells treated with 100 ng/mL PMA for 15 minutes were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was either treated with lambda (λ) phosphatase (1) or left untreated (2-7), blocked with a 3% BSA-TBST buffer for one hour at room temperature, then incubated with the SHP2 [pS^{576}] 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 phosphoserine-containing peptide (4), the phosphopeptide corresponding to SHP2 [pS^{585}] (5), the phosphopeptide corresponding to SHP2 [pS^{591}] (6), or the phosphopeptide immunogen (7). 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 SHP2 [pS^{576}] 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 (Cat. # 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 PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH_2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the PVDF 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) for one hour at room temperature or overnight at 4°C.
12. Incubate the blocked blot with primary antibody at a 1:1,000 starting dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 for two hours at room temperature or overnight at 4°C.
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 (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # 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 Cat. # 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
3 gm Ig-free BSA
0.1 mL Tween 20

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