Rabbit (polyclonal) Anti-Insulin Receptor [pY^{1328}] (Human IR Isoform B [pY^{1316}]) Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number: 44-807G (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 pre-adsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated Inuslin Receptor. The final product is generated by affinity chromatography using an Insulin Receptor-derived peptide that is phosphorylated at tyrosine 1328.

Immunogen: The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human Insulin Receptor that contains tyrosine 1328.

Target Summary: Insulin Receptor and Insulin-like Growth Factor Receptor are transmembrane tyrosine kinase receptors that play critical roles in development, cell growth and metabolism. Both receptors are composed of α subunits which contain ligand binding sites, and β subunits which are hormone stimulated, tyrosine-specific protein kinases. The β subunits contain several autophosphorylation sites located in three major domains: the juxtamembrane domain, the activation domain, and the carboxyl domain. Activation of the tyrosine kinase activity of IR results in immediate autophosphorylation of six tyrosine residues followed by the serine sites in the β subunit (98 kDa) and tyrosine and serine phosphorylation of the intracellular substrates of the receptor. Tyrosine autophosphorylation within the activation loop is correlated with full activation of the receptor. The C terminus of the β-subunit of the Insulin Receptor contains two tyrosine residues in the distal region (tyrosine 1328 and tyrosine 1334) that are phosphorylated in response to insulin. Of these, tyrosine 1328 is not conserved in the IGF-1 receptor and is replaced by phenylalanine 1310. Phosphorylated tyrosine 1328 mediates PI3Kinase activation and plays an important role in elucidating the differential metabolic signaling of IR in response to insulin stimulation.

Reactivity: Human Insulin Receptor when phosphorylated at tyrosine 1328, and the B isoform of human Insulin Receptor when phosphorylated at tyrosine 1316. This antibody is not reactive with Insulin Receptor when phosphorylated at tyrosine 1334, as determined by peptide competition analysis.

Applications: The antibody has been used for Western blotting applications.

Suggested Working Dilutions: For Western blotting applications, we recommend using the antibody at a 1:1000 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.

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

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

Positive Controls Used: Insulin–treated CHO-T cells expressing Human Insulin Receptor.

Related Products:

Antibodies:

IR/IGF1R [pY^{972}], Cat. # 44-800G	Akt [pS^{473}], Cat. # 44-621G
IR/IGF1R [pY^{1158}], Cat. # 44-802G	Akt [pT^{308}], Cat. # 44-602G
IR/IGF1R [pYpY$^{1162/1163}$], Cat. # 44-804G	PRAS40 [pT^{246}], Cat. # 44-1100G
IR/IGF1R [pYpYpY$^{1158/1162/1163}$], Cat. # 44-806G	ERK1&2/MAPK [pTpY$^{185/187}$], Cat. # 44-680G
IRS1 [pS^{616}], Cat. # 44-550G	AS160 [pT^{642}] , Cat # 44-1071G
IRS1 [pS^{312}], Cat. # 44-814G	AMPKα [pT^{172}], Cat. # 44-1150G
IRS1 [pY^{612}], Cat. # 44-816G	GS muscle [pSpS$^{641/645}$], Cat. # 44-1092G
IRS1 [pY^{896}], Cat. # 44-818G	GSK-3β [pS^{9}], Cat. # 44-600G

References:

Federico, L.M., et al. (2006) Intestinal insulin resistance and aberrant production of apolipoprotein B48 lipoproteins in an animal model of insulin resistance and metabolic dyslipidemia: evidence for activation of protein tyrosine phosphatase-1B, extracellular signal-related kinase, and sterol regulatory element-binding protein-1c in the fructose-fed hamster intestine. Diabetes 55(5):1316-1326.

Kusari, A.B., et al. (2000) Substitution of two insulin receptor carboxy-terminal tyrosines with phenylalanine impairs the expression of MAP kinase phosphatase-1 (MKP-1) mRNA. Mol. Cell. Biochem. 211(1-2):27-37.

Soni, P., et al. (2000) The differential effects of pp120 (Ceacam 1) on the mitogenic action of insulin and insulin-like growth factor 1 are regulated by the nonconserved tyrosine 1316 in the insulin receptor. Mol. Cell Biol. 20(11):3896-3905.

Blakesley, V.A., et al. (1996) Tumorigenic and mitogenic capacities are reduced in transfected fibroblasts expressing mutant insulin-like growth factor (IGF)-I receptors. The role of tyrosine residues 1250, 1251, and 1316 in the carboxy-terminus of the IGF-I receptor. Endocrinology 137(2):410-417.

Ando, A., et al. (1992) Enhanced insulin-induced mitogenesis and mitogen-activated protein kinase activities in mutant insulin receptors with substitution of two COOH-terminal tyrosine autophosphorylation sites by phenylalanine. J. Biol. Chem. 267(18):12788-12796.

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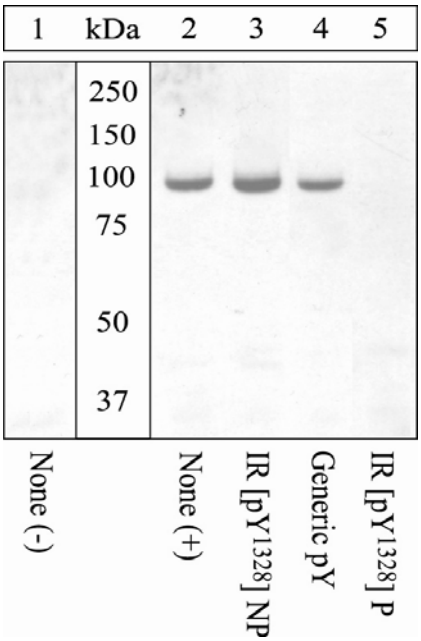

Antibody Specificity

Lysates prepared from CHO-T cells left untreated (lane 1), or treated with insulin (100 nM, 5 minutes; lanes 2-5) were resolved on a 10% polyacrylamide gel and transferred to PVDF. The membrane was blocked with a 3% BSA-TBST buffer for one hour at room temperature, and then incubated with the Insulin Receptor [pY^{1328}] antibody for two hours at room temperature in 3% BSA-TBST buffer, following prior incubation with: no peptide (1), the non-phosphopeptide corresponding to the phosphopeptide immunogen (2), a generic phosphotyrosine-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 the β subunit (98 kDa) of Insulin Receptor was induced by insulin treatment and was blocked only by the phosphopeptide corresponding to IR [pY^{1328}], indicating that the signal is phosphorylation site-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 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 1:1000 dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for one hour 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 (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
5 gm Ig-free BSA
0.1 mL Tween 20

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