Overview

Product name: Anti-Insulin Receptor (phospho Y972) antibody

Description: Rabbit polyclonal to Insulin Receptor (phospho Y972)

Host species: Rabbit

Specificity: In some cell systems ab5678 has been shown to cross-react with IGF1R pY950 (75% homologous).

Tested applications: Suitable for: ICC/IF, IP, WB

Species reactivity: Reacts with: Mouse, Rat, Human

Immunogen: Synthetic peptide (Human). Synthetic phosphopeptide derived from the region of the human Insulin Receptor that contains tyrosine 972 (as numbered according to Ebina, et al. (tyrosine 960 according to Ullrich, et al.).

Positive control: CHO-T cells transfected with a vector encoding the human insulin receptor and stimulated with insulin, and 3T3-L1 adipocytes +/- insulin stimulation.

General notes: Biological actions of insulin are mediated by the Insulin Receptor (IR), a receptor tyrosine kinase that regulates multiple signaling pathways through activation of a series of phosphorylation cascades. The IR is a heterotetrameric protein consisting of two ligand-binding alpha subunits and two beta subunits that each contain a tyrosine kinase domain. Insulin binding to the extracellular domain leads to autophosphorylation of the receptor and activation of the intrinsic tyrosine kinase activity, which allows appropriate substrates to be phosphorylated. Tyrosine 972 is in the juxtamembrane Asn-Pro- Glu-Tyr (NPEY) motif. Phosphorylation of IR tyrosine 972 is required for the binding and/or phosphorylation of the adapter protein Shc, the PTB domain, IRS-1, PI3 kinase, and the Suppressor of Cytokine Signaling (SOCS).

Properties

Form: Liquid

Storage instructions: Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

Storage buffer: Preservative: 0.05% Sodium Azide

Constituents: 0.1% BSA, PBS, pH 7.4

Purity: Immunogen affinity purified

Purification notes: 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 Insulin.
Receptor (IR). The final product is generated by affinity chromatography using an IR-derived peptide phosphorylated at tyrosine 972.

**Primary antibody notes**

Biological actions of insulin are mediated by the Insulin Receptor (IR), a receptor tyrosine kinase that regulates multiple signaling pathways through activation of a series of phosphorylation cascades. The IR is a heterotetrameric protein consisting of two ligand-binding alpha subunits and two beta subunits that each contain a tyrosine kinase domain. Insulin binding to the extracellular domain leads to autophosphorylation of the receptor and activation of the intrinsic tyrosine kinase activity, which allows appropriate substrates to be phosphorylated. Tyrosine 972 is in the juxtamembrane Asn-Pro-Glu-Tyr (NPEY) motif. Phosphorylation of IR tyrosine 972 is required for the binding and/or phosphorylation of the adapter protein Shc, the PTB domain, IRS-1, PI3 kinase, and the Suppressor of Cytokine Signaling (SOCS).

**Clonality**

Polyclonal

**Isotype**

IgG

**Applications**

Our Abpromise guarantee covers the use of ab5678 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>1/100 - 1/500.</td>
<td></td>
</tr>
<tr>
<td>IP</td>
<td>Use at an assay dependent concentration. PubMed: 17264162</td>
<td></td>
</tr>
<tr>
<td>WB</td>
<td>Use a concentration of 0.1 - 1 µg/ml. Detects a band of approximately 110 kDa</td>
<td></td>
</tr>
</tbody>
</table>

**Target**

Receptor tyrosine kinase which mediates the pleiotropic actions of insulin. Binding of insulin leads to phosphorylation of several intracellular substrates, including, insulin receptor substrates (IRS1, 2, 3, 4), SHC, GAB1, CBL and other signaling intermediates. Each of these phosphorylated proteins serve as docking proteins for other signaling proteins that contain Src-homology-2 domains (SH2 domain) that specifically recognize different phosphotyrosines residues, including the p85 regulatory subunit of PI3K and SHP2. Phosphorylation of IRSs proteins lead to the activation of two main signaling pathways: the PI3K-AKT/PKB pathway, which is responsible for most of the metabolic actions of insulin, and the Ras-MAPK pathway, which regulates expression of some genes and cooperates with the PI3K pathway to control cell growth and differentiation. Binding of the SH2 domains of PI3K to phosphoryrosines on IRS1 leads to the activation of PI3K and the generation of phosphatidylinositol-(3, 4, 5)-triphosphate (PIP3), a lipid second messenger, which activates several PIP3-dependent serine/threonine kinases, such as PDPK1 and subsequently AKT/PKB. The net effect of this pathway is to produce a translocation of the glucose transporter SLC2A4/GLUT4 from cytoplasmic vesicles to the cell membrane to facilitate glucose transport. Moreover, upon insulin stimulation, activated AKT/PKB is responsible for: anti-apoptotic effect of insulin by inducing phosphorylation of BAD; regulates the expression of gluconeogenic and lipogenic enzymes by controlling the activity of the winged helix or forhead (FOX) class of transcription factors. Another pathway regulated by PI3K-AKT/PKB activation is mTORC1 signaling pathway which regulates cell growth and metabolism and integrates signals.
from insulin. AKT mediates insulin-stimulated protein synthesis by phosphorylating TSC2 thereby activating mTORC1 pathway. The Ras/RAF/MAP2K/MAPK pathway is mainly involved in mediating cell growth, survival and cellular differentiation of insulin. Phosphorylated IRS1 recruits GRB2/SOS complex, which triggers the activation of the Ras/RAF/MAP2K/MAPK pathway. In addition to binding insulin, the insulin receptor can bind insulin-like growth factors (IGF1 and IGFII). Isoform Short has a higher affinity for IGFII binding. When present in a hybrid receptor with IGF1R, binds IGF1. PubMed:12138094 shows that hybrid receptors composed of IGF1R and INSR isoform Long are activated with a high affinity by IGF1, with low affinity by IGF2 and not significantly activated by insulin, and that hybrid receptors composed of IGF1R and INSR isoform Short are activated by IGF1, IGF2 and insulin. In contrast, PubMed:16831875 shows that hybrid receptors composed of IGF1R and INSR isoform Long and hybrid receptors composed of IGF1R and INSR isoform Short have similar binding characteristics, both bind IGF1 and have a low affinity for insulin.

**Tissue specificity**

Isoform Long and isoform Short are predominantly expressed in tissue targets of insulin metabolic effects: liver, adipose tissue and skeletal muscle but are also expressed in the peripheral nerve, kidney, pulmonary alveoli, pancreatic acini, placenta vascular endothelium, fibroblasts, monocytes, granulocytes, erythrocytes and skin. Isoform Short is preferentially expressed in fetal cells such as fetal fibroblasts, muscle, liver and kidney. Found as a hybrid receptor with IGF1R in muscle, heart, kidney, adipose tissue, skeletal muscle, hepatoma, fibroblasts, spleen and placenta (at protein level). Overexpressed in several tumors, including breast, colon, lung, ovary, and thyroid carcinomas.

**Involvement in disease**

- Rabson-Mendenhall syndrome
- Leprechaunism
- Diabetes mellitus, non-insulin-dependent
- Familial hyperinsulinemic hypoglycemia 5
- Insulin-resistant diabetes mellitus with acanthosis nigricans type A

**Sequence similarities**

Belongs to the protein kinase superfamily. Tyr protein kinase family. Insulin receptor subfamily. Contains 3 fibronectin type-III domains. Contains 1 protein kinase domain.

**Domain**

The tetrameric insulin receptor binds insulin via non-identical regions from two alpha chains, primarily via the C-terminal region of the first INSR alpha chain. Residues from the leucine-rich N-terminus of the other INSR alpha chain also contribute to this insulin binding site. A secondary insulin-binding site is formed by residues at the junction of fibronectin type-III domain 1 and 2.

**Post-translational modifications**

After being transported from the endoplasmic reticulum to the Golgi apparatus, the single glycosylated precursor is further glycosylated and then cleaved, followed by its transport to the plasma membrane. Autophosphorylated on tyrosine residues in response to insulin. Phosphorylation of Tyr-999 is required for binding to IRS1, SHC1 and STAT5B. Dephosphorylated by PTPRE at Tyr-999, Tyr-1185, Tyr-1189 and Tyr-1190. Dephosphorylated by PTPRF and PTPN1. Dephosphorylated by PTPN2; down-regulates insulin-induced signaling.

**Cellular localization**

Cell membrane.
Immunofluorescence analysis of Insulin Receptor (phospho Y972) was done on 70% confluent log phase MCF7 cells with insulin treatment (100nM for 5 min). The cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.25% Triton™ X-100 for 10 minutes, and blocked with 5% BSA for 1 hour at room temperature. The cells were labeled with Anti-Insulin Receptor (phospho Y972) antibody (ab5678) at 2µg/mL in 1% BSA and incubated for 3 hours at room temperature and then labeled with Alexa Fluor 488® Goat Anti-Rabbit IgG Secondary Antibody at a dilution of 1/400 for 30 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with DAPI. F-actin (Panel c: red) was stained with Alexa Fluor 594® Phalloidin. Panel d is a merged image showing membrane localization. Panel e is untreated MCF7 cells. Panel f shows no primary antibody control. The images were captured at 20X magnification.

Upregulation and Antibody-Peptide Competition. Extracts of CHO-T cells transfected with an insulin receptor-containing vector and left unstimulated (1) or stimulated with 50 nM insulin for 5 minutes (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 Anti-Insulin Receptor (phospho Y972) antibody (ab5678) for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 2), the non-phosphorylated peptide 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. The data show that only the phosphopeptide corresponding to Insulin Receptor (phospho Y972) completely blocks the antibody signal, demonstrating the specificity of the antibody. The data also show up-regulation of the signal upon stimulation with insulin in this cell system.
Peptide Competition: Extracts prepared from CHO-T cells stimulated with insulin were resolved by SDS-PAGE on a 10% Tris-glycine gel, and transferred to PVDF. Membranes were incubated with 0.50 µg/mL ab5678 antibody, following prior incubation in the presence of: the phosphopeptide immunogen (1), the non-phosphopeptide corresponding to the Insulin Receptor phosphopeptide (2), a generic phosphotyrosine containing peptide (3), or, the absence of the phosphopeptide immunogen (4). After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG alkaline phosphatase and bands were detected using the Tropix WesternStar detection method. The data show detection of a single band corresponding to the α-subunit (~95 kDa) of the Insulin Receptor. The data also show that only the phosphopeptide corresponding to this site blocks the antibody signal, demonstrating the specificity of the ab5678 antibody for this epitope. Insulin Stimulation Extracts pre

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors