


Anti-Insulin Receptor (phospho Y972) antibody ab5678

★ ★ ★ ★ ★ [2 Abreviews](#) [10 References](#) [2 Images](#)

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, WB
Species reactivity	Reacts with: Human Predicted to work with: Mouse, Rat 
Immunogen	Synthetic peptide corresponding to Human Insulin Receptor (phospho Y972).
Positive control	IF: Insulin treated MCF7 cells. WB: CHO-T (Chinese hamster ovary cell line) cells transfected with a vector encoding the human insulin receptor and stimulated with insulin.
General notes	<p>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).</p> <p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As</p>

Properties

Form	Liquid
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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	pH: 7.30 Preservative: 0.05% Sodium azide Constituents: PBS, 1% BSA, 50% Glycerol
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

The Abpromise guarantee 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.

Application	Abreviews	Notes
ICC/IF		1/100 - 1/500.
WB	★ ★ ★ ★ ★ (2)	1/1000. Detects a band of approximately 110 kDa.

Target

Function 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 phosphotyrosines 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 forkhead (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 (IGF I and IGF II). Isoform Short has a higher affinity for IGF II binding. When present in a hybrid receptor with IGF 1R, binds IGF 1. PubMed:12138094 shows that hybrid receptors composed of IGF 1R and INSR isoform Long are activated with a high affinity by IGF 1, with low affinity by IGF 2 and not significantly activated by insulin, and that hybrid receptors composed of IGF 1R and INSR isoform Short are activated by IGF 1, IGF 2 and insulin. In contrast, PubMed:16831875 shows that hybrid receptors composed of IGF 1R and INSR isoform Long and hybrid receptors composed of IGF 1R and INSR isoform Short have similar binding characteristics, both bind IGF 1 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 IGF 1R 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

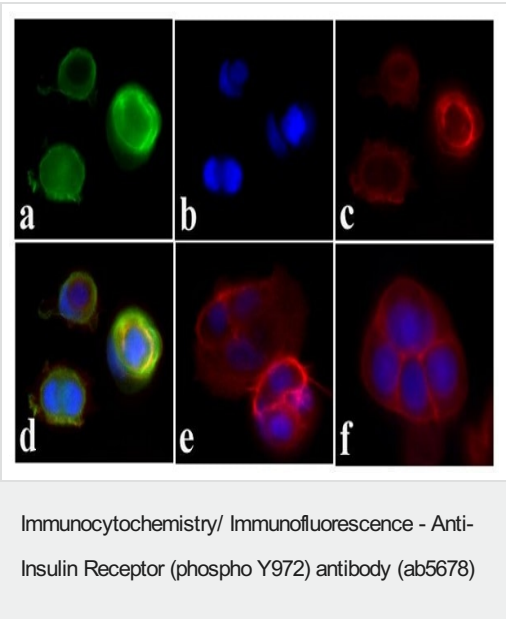
The tetrameric insulin receptor binds insulin via non-identical regions from two alpha chains, primarily via the C-terminal region of the first INSR alpha chain. Residues from the leucine-rich N-terminus of the other INSR alpha chain also contribute to this insulin binding site. A secondary insulin-binding 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.

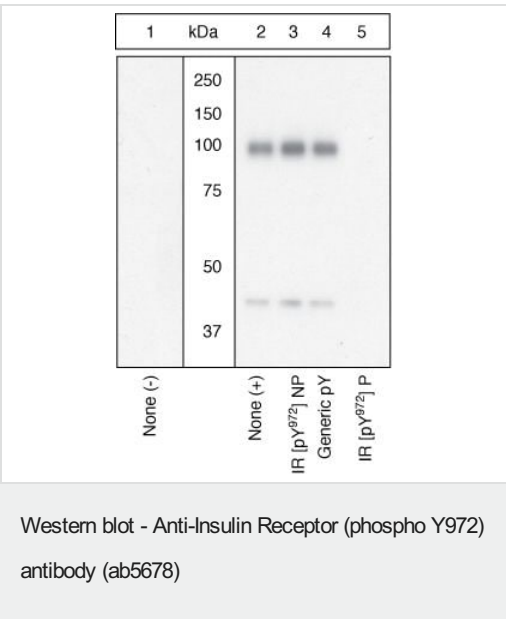
Cellular localization

Cell membrane.



Immunofluorescence analysis of insulin treated MCF7 cells labelling Insulin Receptor (phospho Y972) (Panel a: green) using ab5678 at 2µg/mL in 1% BSA for 3 hours at room temperature, followed by Alexa Fluor 488® Goat Anti-Rabbit IgG Secondary Antibody at 1/400 dilution for 30 minutes at room temperature. Panel b: Nuclei were stained with DAPI (blue). Panel c: F-actin was stained with Alexa Fluor 594® Phalloidin (red). Panel d: Merged image showing membrane localization. Panel e: Untreated MCF7 cells. Panel f: Control, no primary antibody. The images were captured at 20X magnification.

Prior antibody incubation, MCF7 (human breast adenocarcinoma cell line) 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, followed by treatment with 100nM of insulin for 5 min. Assay was done on 70% confluent log phase MCF7 cells.



All lanes : Anti-Insulin Receptor (phospho Y972) antibody (ab5678) at 1/1000 dilution (2 hours at room temperature in a 3% BSA-TBST buffer)

Lane 1 : Unstimulated (-), CHO-T transfected with insulin receptor containing vector whole cell extract with 5% BSA-TBST buffer for one hour at room temperature

Lanes 2-5 : Stimulated (+) with 50 nM insulin for 5 minutes, CHO-T transfected with insulin receptor containing vector whole cell extract with 5% BSA-TBST buffer for one hour at room temperature

Secondary

All lanes : Goat F (ab')₂ anti-rabbit IgG HRP conjugate

Upregulation and Antibody-Peptide Competition:

Prior primary antibody incubation:

- 1 and 2 - no peptide;
- 3 - non-phosphorylated peptide corresponding to the phosphopeptide immunogen;
- 4 - generic phosphotyrosine-containing peptide;
- 5 - phosphopeptide immunogen.

SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF.

The data show that only the phosphopeptide corresponding to ab5678 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.

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