abcam

Product datasheet

Anti-Insulin Receptor (phospho Y1361) antibody ab60946

★★★★★★ 5 Abreviews 25 References 7 Images

Overview

Properties

Product name	Anti-Insulin Receptor (phospho Y1361) antibody	
Description	Rabbit polyclonal to Insulin Receptor (phospho Y1361)	
Host species	Rabbit	
Tested applications	Suitable for: IHC-P, ELISA, WB	
Species reactivity	Reacts with: Human, Chinese hamster	
	Predicted to work with: Mouse	
Immunogen	Synthetic peptide corresponding to Human Insulin Receptor aa 1300-1400 (phospho Y1361). The immunogen is a synthesized peptide derived from human Insulin Receptor around the phosphorylation site of Tyr1361. Database link: P06213	
Positive control	WB: 293 cell lysate, CHO treated with 0.01 U/mL Insulin for 15 minutes lysate, 293 treated with 0.01 U/mL Insulin for 15 minutes lysate. IHC-P: Human spleen and breast carcinoma; Rat brain.	
General notes	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.	
	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	

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 50% Glycerol, 0.87% Sodium chloride
	PBS without Mg2+ and Ca2+

Purity	Immunogen affinity purified
Purification notes	ab60946 was affinity-purified from rabbit antiserum by affinity-chromatography using epitope- specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatography using non-phosphopeptide corresponding to the phosphorylation site.
Clonality	Polyclonal
lsotype	lgG

Applications

 The Abpromise guarantee
 Our Abpromise guarantee
 covers the use of ab60946 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
IHC-P	★ ★ ★ ★ ★ <u>(1)</u>	1/50 - 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ELISA		1/10000.
WB	****(2)	1/500 - 1/1000. Detects a band of approximately 90 kDa (predicted molecular weight: 156 kDa).

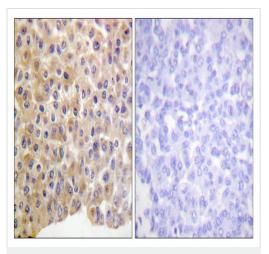
Target

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: antiapoptotic 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 (IGFI and IGFII).

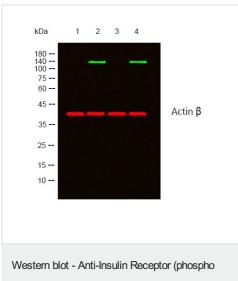
	Isoform Short has a higher affinity for IGFII binding. When present in a hybrid receptor with IGF1R, binds IGF1. PubMed:12138094 shows that hybrid receptors composed of IGF1R and INSR isoform Long are activated with a high affinity by IGF1, with low affinity by IGF2 and not significantly activated by 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.

Images



Immunohistochemistry analysis of paraffin-embedded human breast carcinoma stained with ab60946. The image on the right is blocked with the phospho peptide.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Insulin Receptor (phospho Y1361) antibody (ab60946)



Y1361) antibody (ab60946)

All lanes : Anti-Insulin Receptor (phospho Y1361) antibody (ab60946) at 1/1000 dilution

- Lane 1 : CHO untreated lysate
- Lane 2 : CHO treated with 0.01 U/mL Insulin for 15 minutes lysate
- Lane 3: 293 untreated lysate
- Lane 4 : 293 treated with 0.01 U/mL Insulin for 15 minutes lysate

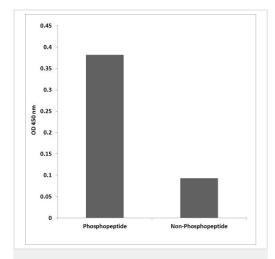
Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG (H+L) HRP at 1/10000 dilution

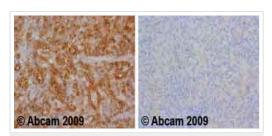
Predicted band size: 156 kDa

Loading control: Beta Actin



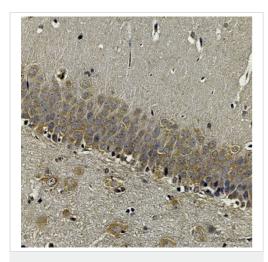
ab60946 (1:10000) antibody detects endogenous levels of Insulin Receptor only when phosphorylated at Tyr1361.

ELISA - Anti-Insulin Receptor (phospho Y1361) antibody (ab60946)

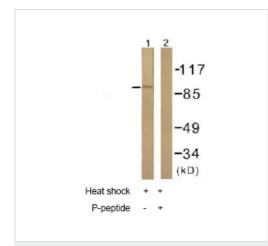


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Insulin Receptor (phospho Y1361) antibody (ab60946) Ab60946 staining human spleen. Staining is localized to the membrane, with some light staining in the cytoplasm. Left panel: with primary antibody at 1 ug/ml. Right panel: isotype control.

Sections were stained using an automated system (Dako PT Link), at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 antigen retrieval buffer, citrate pH 6.0. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be requ



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Insulin Receptor (phospho Y1361) antibody (ab60946) This image is courtesy of an anonymous Abreview

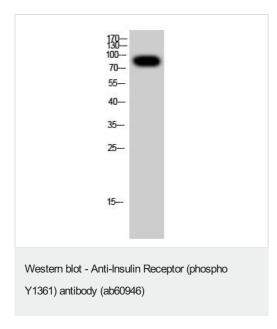


Western blot - Anti-Insulin Receptor (phospho Y1361) antibody (ab60946) ab60946 staining Insulin Receptor in Rat brain tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed with paraformaldehyde and blocked with 8% Milk for 30 minutes at 37°C; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/100 in diluent) for 18 hours at 4°C. A detection reagent was used to detect antibody staining.

All lanes : Anti-Insulin Receptor (phospho Y1361) antibody (ab60946) at 1/500 dilution

Lane 1 : 293 cell extracts treated with Heat shock Lane 2 : 293 cell extracts treated with Heat shock with immunising peptide

Predicted band size: 156 kDa **Observed band size:** 90 kDa



Anti-Insulin Receptor (phospho Y1361) antibody (ab60946) (Antiinsulin receptor (phospho Y1361) antibody) + 293 cell lysate

Predicted band size: 156 kDa

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