# abcam

# Product datasheet

# Anti-Insulin Receptor antibody ab137747

7 References 7 Images

Overview

Product name Anti-Insulin Receptor antibody

**Description** Rabbit polyclonal to Insulin Receptor

Host species Rabbit

Tested applications Suitable for: WB, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Recombinant fragment, corresponding to a region within internal sequence amino acids 939-

1347 of Human Insulin Receptor

Positive control WB: Wild-type HepG2, HeLa, HEK-293T, NIH-3T3 and PC-12 cell lysates. IHC-P: Human gastric

cancer and mouse prostate tissues.

**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

**Properties** 

Form Liquid

**Storage instructions** Shipped at 4°C. Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

Storage buffer pH: 7.00

Preservative: 0.025% Proclin 300

Constituents: 79% PBS, 20% Glycerol (glycerin, glycerine)

**Purity** Immunogen affinity purified

**Clonality** Polyclonal

**Isotype** IgG

**Applications** 

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#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab137747 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
WB		1/1000 - 1/10000. Predicted molecular weight: 156 kDa.
IHC-P		Use at an assay dependent concentration. <b>Antigen Retrieval:</b> Trilogy <sup>™</sup> (EDTA based, pH 8.0) buffer, 15min.

#### **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.

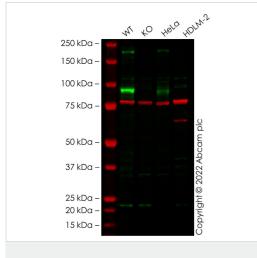
Autophosphorylated on tyrosine residues in response to insulin. Phosphorylation of Tyr-999 is required for binding to IRS1, SHC1 and STAT5B. Dephosphorylated by PTPRE at Tyr-999, Tyr-1185, Tyr-1189 and Tyr-1190. Dephosphorylated by PTPRF and PTPN1. Dephosphorylated by

PTPN2; down-regulates insulin-induced signaling.

**Cellular localization** 

Cell membrane.

#### **Images**



Western blot - Anti-Insulin Receptor antibody (ab137747)

**All lanes :** Anti-Insulin Receptor antibody (ab137747) at 1/1000 dilution

Lane 1: Wild-type HepG2 cell lysate

Lane 2: INSR knockout HepG2 cell lysate

Lane 3 : HeLa cell lysate
Lane 4 : HDLM-2 cell lysate

Lysates/proteins at 20 µg per lane.

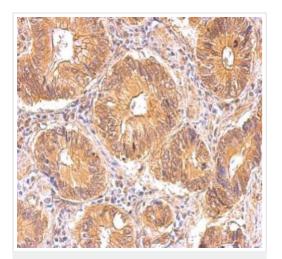
# Secondary

**All lanes :** Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

**Predicted band size:** 156 kDa **Observed band size:** 200,85 kDa

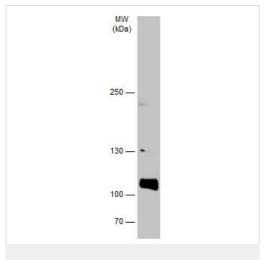
False colour image of Western blot: Anti-Insulin Receptor antibody staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab137747 was shown to bind specifically to Insulin Receptor. A band was observed at 85/200 kDa in wild-type HepG2 cell lysates with no signal observed at this size in INSR knockout cell line ab282827 (knockout cell lysate ab283051). Pro-form of INSR is observed at 200 kDa and the mature form observed at 90 kDa. To generate this image, wild-type and INSR knockout HepG2 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



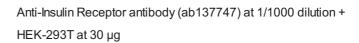
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Insulin Receptor antibody (ab137747)

Immunohistochemical analysis of paraffin-embedded human gastric cancer tissue labelling INSR protein at cytosol with ab137747 at 1/500 dilution

**Antigen Retrieval:** Trilogy™ (EDTA based, pH 8.0) buffer, 15min.



Western blot - Anti-Insulin Receptor antibody (ab137747)

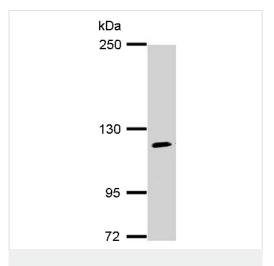


# Secondary

HRP-conjugated anti-rabbit IgG antibody

Predicted band size: 156 kDa

5% SDS PAGE

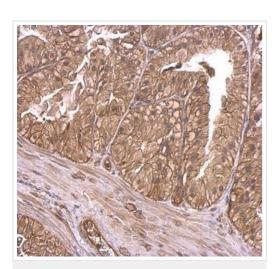


Western blot - Anti-Insulin Receptor antibody (ab137747)

Anti-Insulin Receptor antibody (ab137747) at 1/10000 dilution + HeLa whole cell lysate at 30  $\mu g$ 

Predicted band size: 156 kDa

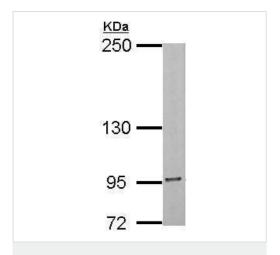
5% SDS PAGE



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Insulin Receptor antibody (ab137747)

Immunohistochemical analysis of paraffin-embedded mouse prostate tissue labelling Insulin receptor protein at membrane with ab137747 at 1/500 dilution.

**Antigen Retrieval:** Trilogy™ (EDTA based, pH 8.0) buffer, 15min.



Western blot - Anti-Insulin Receptor antibody (ab137747)

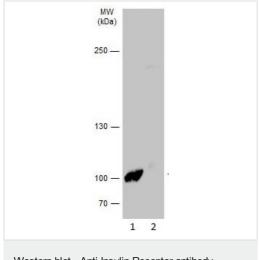
Anti-Insulin Receptor antibody (ab137747) at 1/1000 dilution + NIH-3T3 at 30  $\mu g$ 

#### **Secondary**

HRP-conjugated anti-rabbit IgG antibody

Predicted band size: 156 kDa

5% SDS PAGE



dilution

All lanes: Anti-Insulin Receptor antibody (ab137747) at 1/1000

Lane 1: PC-12 Lane 2: Rat2

Lysates/proteins at 50 µg per lane.

**Secondary** 

All lanes: HRP-conjugated anti-rabbit IgG antibody

Predicted band size: 156 kDa

Western blot - Anti-Insulin Receptor antibody (ab137747)

5% SDS PAGE

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

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