abcam

Product datasheet

Anti-IGF1 Receptor antibody [EPR23027-80] ab263907

KO VALIDATED Recombinant RabMAb

6 Images

Overview

Product name	Anti-IGF1 Receptor antibody [EPR23027-80]
Description	Rabbit monoclonal [EPR23027-80] to IGF1 Receptor
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, Flow Cyt, ICC/IF **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment within Human IGF1 Receptor aa 1-750. The exact sequence is proprietary. Database link: P08069
Positive control	WB: A431, HepG2, HeLa and MDA-MB-231 lysates. ICC/IF: MCF7 cells. Flow Cyt: MCF7 cells. IP: MCF7 cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information see here. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents. Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough. Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility. We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee. In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for. We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

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, as well as customer reviews and Q&As.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23027-80
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab263907** 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. Detects a band of approximately 130, 200 kDa (predicted molecular weight: 154 kDa).
IP		1/30.
Flow Cyt		1/500.
ICC/IF		1/100.

Application notes	Is unsuitable for IHC-P.

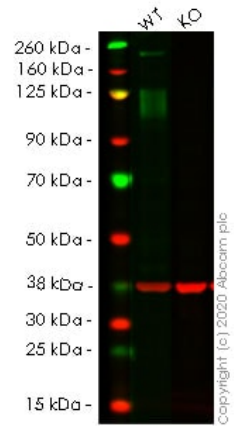
Target

Function	Receptor tyrosine kinase which mediates actions of insulin-like growth factor 1 (IGF1). Binds IGF1 with high affinity and IGF2 and insulin (INS) with a lower affinity. The activated IGF1R is involved in cell growth and survival control. IGF1R is crucial for tumor transformation and survival of malignant cell. Ligand binding activates the receptor kinase, leading to receptor autophosphorylation, and tyrosines phosphorylation of multiple substrates, that function as signaling adapter proteins including, the insulin-receptor substrates (IRS1/2), Shc and 14-3-3 proteins. Phosphorylation of IRSs proteins lead to the activation of two main signaling pathways: the PI3K-AKT/PKB pathway and the Ras-MAPK pathway. The result of activating the MAPK pathway is increased cellular proliferation, whereas activating the PI3K pathway inhibits apoptosis and stimulates protein synthesis. Phosphorylated IRS1 can activate the 85 kDa regulatory subunit of PI3K (PIK3R1), leading to activation of several downstream substrates, including protein AKT/PKB. AKT phosphorylation, in turn, enhances protein synthesis through mTOR activation and triggers the antiapoptotic effects of IGFIR through phosphorylation and inactivation of BAD. In parallel to PI3K-driven signaling, recruitment of Grb2/SOS by phosphorylated IRS1 or Shc leads to recruitment of Ras and activation of the ras-MAPK pathway. In addition to these two main signaling pathways IGF1R signals also through the Janus kinase/signal transducer and activator of transcription pathway (JAK/STAT). Phosphorylation of JAK proteins can lead to phosphorylation/activation of signal transducers and activators of transcription (STAT) proteins. In particular activation of STAT3, may be essential for the transforming activity of IGF1R. The JAK/STAT pathway activates gene transcription and may be responsible for the transforming activity. JNK kinases can also be activated by the IGF1R. IGF1 exerts inhibiting activities on JNK activation via phosphorylation and inhibition of MAP3K5/ASK1, which is able to directly associate with the IGF1R. When present in a hybrid receptor with INSR, 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	Found as a hybrid receptor with INSR in muscle, heart, kidney, adipose tissue, skeletal muscle, hepatoma, fibroblasts, spleen and placenta (at protein level). Expressed in a variety of tissues. Overexpressed in tumors, including melanomas, cancers of the colon, pancreas prostate and kidney.
Involvement in disease	Insulin-like growth factor 1 resistance
Sequence similarities	Belongs to the protein kinase superfamily. Tyr protein kinase family. Insulin receptor subfamily. Contains 4 fibronectin type-III domains. Contains 1 protein kinase domain.
Post-translational modifications	Autophosphorylated on tyrosine residues in response to ligand binding. Autophosphorylation occurs in trans, i.e. one subunit of the dimeric receptor phosphorylates tyrosine residues on the other subunit. Autophosphorylation occurs in a sequential manner; Tyr-1165 is predominantly phosphorylated first, followed by phosphorylation of Tyr-1161 and Tyr-1166. While every single phosphorylation increases kinase activity, all three tyrosine residues in the kinase activation loop (Tyr-1165, Tyr-1161 and Tyr-1166) have to be phosphorylated for optimal activity. Can be autophosphorylated at additional tyrosine residues (in vitro). Autophosphorylated is followed by phosphorylation of juxtamembrane tyrosines and C-terminal serines. Phosphorylation of Tyr-980 is required for IRS1- and SHC1-binding. Phosphorylation of Ser-1278 by GSK-3beta restrains kinase activity and promotes cell surface expression, it requires a priming phosphorylation at Ser-1282. Dephosphorylated by PTPN1. Polyubiquitinated at Lys-1168 and Lys-1171 through both 'Lys-48' and 'Lys-29' linkages, promoting receptor endocytosis and subsequent degradation by the proteasome. Ubiquitination

is facilitated by pre-existing phosphorylation.
Sumoylated with SUMO1.
Controlled by regulated intramembrane proteolysis (RIP). Undergoes metalloprotease-dependent constitutive ectodomain shedding to produce a membrane-anchored 52 kDa C-Terminal fragment which is further processed by presenilin gamma-secretase to yield an intracellular 50 kDa fragment.

Cellular localization Cell membrane.

Images



Western blot - Anti-IGF1 Receptor antibody [EPR23027-80] (ab263907)

All lanes : Anti-IGF1 Receptor antibody [EPR23027-80] (ab263907) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : IGF1R knockout HeLa cell lysate

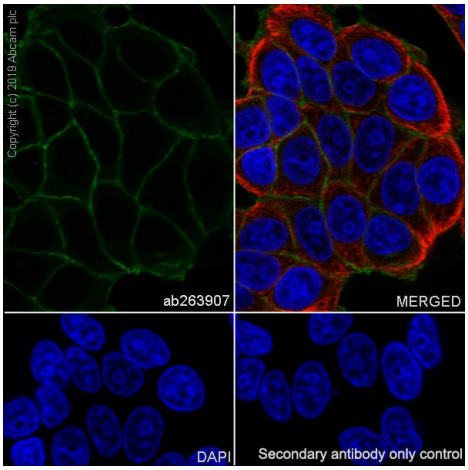
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 154 kDa
Observed band size: 100 kDa
why is the actual band size different from the predicted?

Lanes 1- 2: Merged signal (red and green). Green - ab263907 observed at 100 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

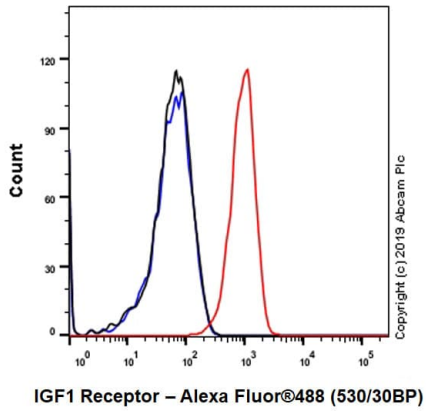
ab263907 was shown to react with IGF1 Receptor in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab264801 (knockout cell lysate ab256951) was used. Wild-type HeLa and IGF1R knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab263907 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-IGF1 Receptor antibody [EPR23027-80] (ab263907)

Immunofluorescent analysis of 100% Methanol-fixed MCF7 (human breast adenocarcinoma epithelial cell) cells labelling IGF1 Receptor with ab263907 at 1/100 dilution, followed by ab150077 AlexaFluor®488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing membranous staining in MCF7 cell line. ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

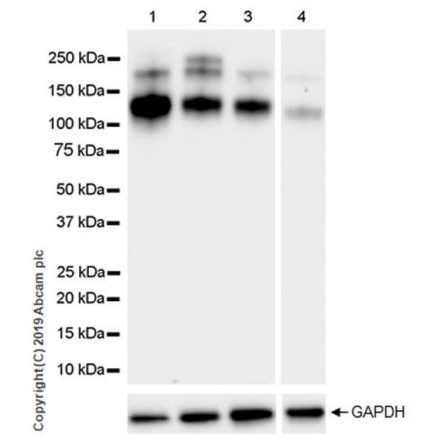
Secondary antibody only control: Secondary antibody is ab150077 AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 dilution.



Flow Cytometry - Anti-IGF1 Receptor antibody [EPR23027-80] (ab263907)

Flow cytometric analysis MCF7 (human breast adenocarcinoma epithelial cell) cells labelling IGF1 Receptor with ab263907 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.

Gated on viable cells.



Western blot - Anti-IGF1 Receptor antibody [EPR23027-80] (ab263907)

All lanes : Anti-IGF1 Receptor antibody [EPR23027-80] (ab263907) at 1/1000 dilution

Lane 1 : A431 (human epidermoid carcinoma epithelial cell) whole cell lysate
Lane 2 : HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 4 : MDA-MB-231 (human breast adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 154 kDa

Observed band size: 130,200 kDa why is the actual band size different from the predicted?

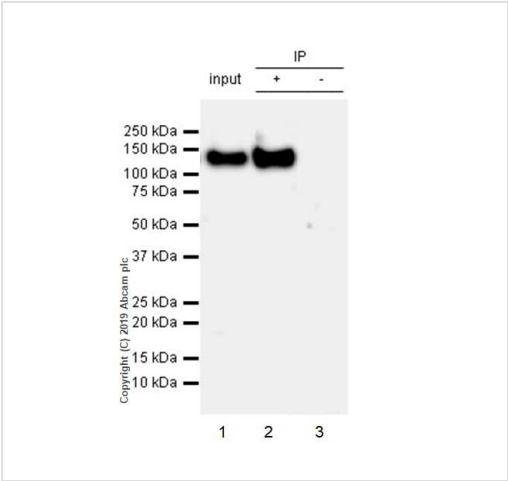
Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 10 seconds.

The expression profile & molecular weight observed is consistent with what has been described in the literature (PMID: 21807868, 28591735).

Note: the bands larger than 200kDa are Pro-IGF1R.

Low expression cell line: MDA-MB-231 (PMID: 28591735).



Immunoprecipitation - Anti-IGF1 Receptor antibody [EPR23027-80] (ab263907)

IGF1 Receptor was immunoprecipitated from 0.35 mg MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate with ab263907 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab263907 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

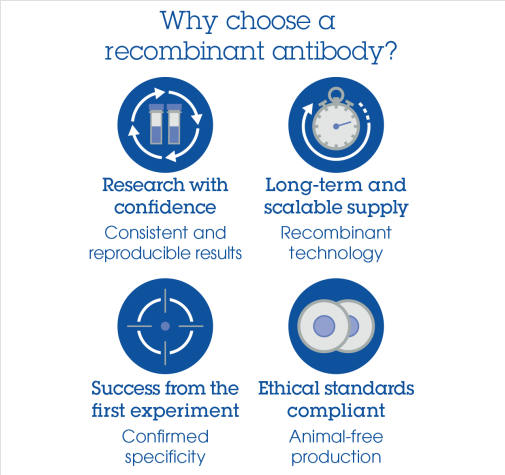
Lane 1: MCF7 (human breast adenocarcinoma epithelial cell) whole cell lysate 10ug

Lane 2: ab263907 IP in MCF7 whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab263907 in MCF7 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 min.



Anti-IGF1 Receptor antibody [EPR23027-80]

(ab263907)

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