abcam

Product datasheet

Anti-IGF1 Receptor antibody [EPR19322] - Low endotoxin, Azide free ab246702

KO VALIDATED Recombinant RabMAb

7 Images

Overview

Product name	Anti-IGF1 Receptor antibody [EPR19322] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EPR19322] to IGF1 Receptor - Low endotoxin, Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IP, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: C2C12, HeLa, 293, MCF7, C6, and NIH/3T3 whole cell lysates; Mouse brain and spleen lysates; Rat brain lysate. ICC/IF: C2C12 and C6 cells. Flow Cyt (intra): C2C12 cells. IP: C2C12 whole cell lysate.
General notes	ab246702 is the carrier-free version of **ab182408**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Purification notes	Endotoxin level is less than 1 EU/ml as determined by the TAL test.
Clonality	Monoclonal
Clone number	EPR19322
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab246702 in the following tested applications.

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

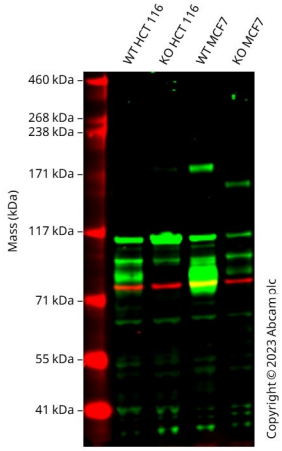
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 100, 200 kDa (predicted molecular weight: 154 kDa).
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

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 [EPR19322] - Low endotoxin, Azide free (ab246702)

All lanes : Anti-IGF1 Receptor antibody [EPR19322] (**ab182408**) at 1/1000 dilution

Lane 1 : Wild-type HCT 116 cell lysate
Lane 2 : IGF1R knockout HCT 116 cell lysate
Lane 3 : Wild-type MCF7 **ab290784** cell lysate
Lane 4 : IGF1R knockout MCF7 **ab287507** cell lysate

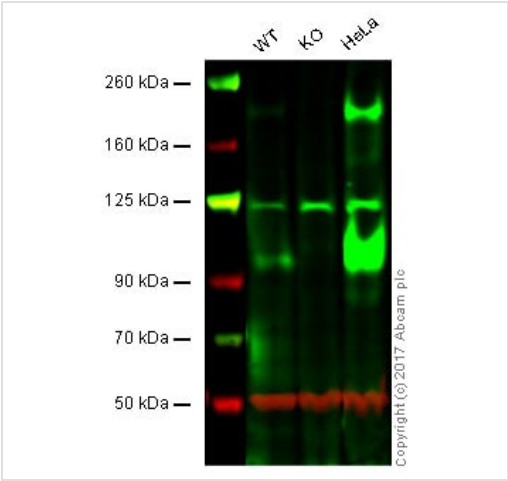
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 154 kDa
Observed band size: 82 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab182408**).

Anti-IGF1R antibody [EPR19322] (**ab182408**) 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, **ab182408** was shown to bind specifically to IGF1R. A band was observed at 82 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in IGF1R knockout cell line. To generate this image, wild-type and IGF1R knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution 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.



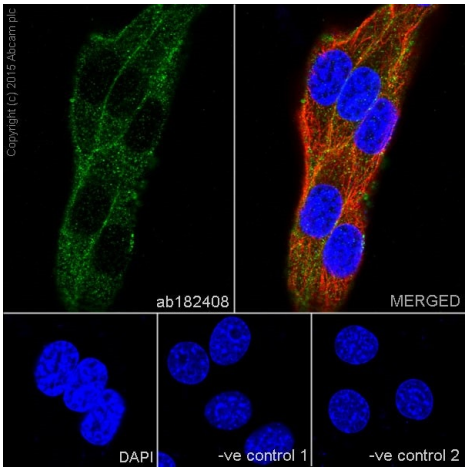
Western blot - Anti-IGF1 Receptor antibody [EPR19322] - Low endotoxin, Azide free (ab246702)

Lane 1: Wild-type HAP1 whole cell lysate (40 µg)
Lane 2: IGF1R knockout HAP1 whole cell lysate (40 µg)
Lane 3: HeLa whole cell lysate (40 µg)

Lanes 1 - 3 Merged signal (red and green). Green - **ab182408** observed at 100 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab182408 was shown to specifically recognize IGF1R in wild-type HAP1 cells along with additional cross-reactve bands. No band was observed when IGF1R knockout samples were examined. Wild-type and IGF1R knockout samples were subjected to SDS-PAGE. Ab182408 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/2000 dilution and 1/10,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab182408**).



Immunocytochemistry/ Immunofluorescence - Anti-IGF1 Receptor antibody [EPR19322] - Low endotoxin, Azide free (ab246702)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized C2C12 (Mouse myoblast cell line) cells labeling IGF1 Receptor with **ab182408** at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous and cytoplasmic staining on C2C12 cell line. The nuclear counterstain is DAPI (blue).

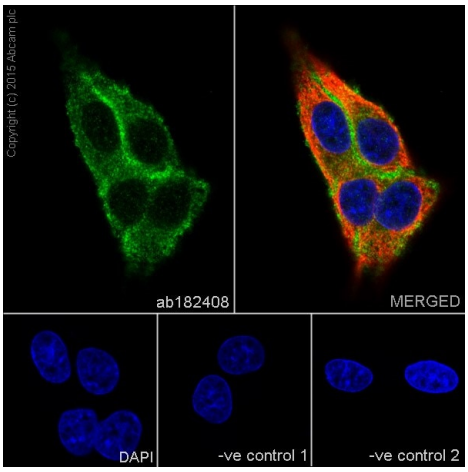
Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: **ab182408** at 1/1000 dilution followed by **ab150120** at 1/1000 dilution.
-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and

sodium azide (**ab182408**).



Immunocytochemistry/ Immunofluorescence - Anti-IGF1 Receptor antibody [EPR19322] - Low endotoxin, Azide free (ab246702)

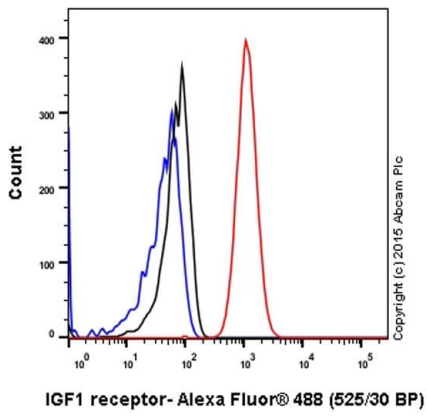
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized C6 (Rat glial tumor cell line) cells labeling IGF1 Receptor with **ab182408** at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous and cytoplasmic staining on C6 cell line. The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: **ab182408** at 1/1000 dilution followed by **ab150120** at 1/1000 dilution.
-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.

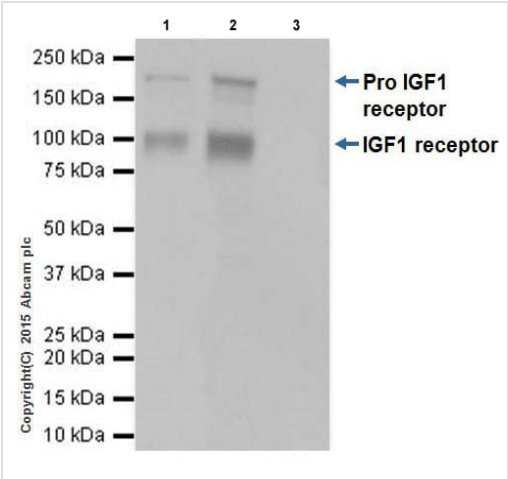
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab182408**).



Flow Cytometry (Intracellular) - Anti-IGF1 Receptor antibody [EPR19322] - Low endotoxin, Azide free (ab246702)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed C2C12 (Mouse myoblast cell line) cells labeling IGF1 Receptor with **ab182408** at 1/80 dilution (red) compared with a Rabbit IgG,monoclonal[EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluor® 488) at 1/500 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab182408**).



Immunoprecipitation - Anti-IGF1 Receptor antibody [EPR19322] - Low endotoxin, Azide free (ab246702)

IGF1 Receptor was immunoprecipitated from 1mg of C2C12 (Mouse myoblast cell line) whole cell lysate with **ab182408** at 1/50 dilution. Western blot was performed from the immunoprecipitate using **ab182408** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: C2C12 whole cell lysate, 10µg (Input).

Lane 2: **ab182408** IP in C2C12 whole cell lysate.

Lane 3: Rabbit IgG,monoclonal [EPR25A] - Isotype Control (**ab172730**) instead of **ab182408** in C2C12 whole cell lysate.

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

Exposure time: 3 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol and sodium azide (**ab182408**).

Anti-IGF1 Receptor antibody [EPR19322] - Low endotoxin, Azide free (ab246702)

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