# abcam

# Product datasheet

# Anti-IGF1 Receptor antibody [EPR19322] ab182408



★★★★ 1 Abreviews 46 References 画像数 11

#### 製品の概要

製品名 Anti-IGF1 Receptor antibody [EPR19322]

製品の詳細 Rabbit monoclonal [EPR19322] to IGF1 Receptor

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), WB, ICC/IF, IP

種交差性 交差種: Mouse, Rat, Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール 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.

特記事項 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**.

#### 製品の特性

製品の状態 Liquid

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

ポリ/モノ モノクローナル クローン名 EPR19322

#### アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab182408の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/80.
WB	<b>★★★★☆ (1)</b>	1/1000. Detects a band of approximately 100,200 kDa (predicted molecular weight: 156 kDa).
ICC/IF		1/1000.
IP		1/50.

#### ターゲット情報

#### 機能

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 PI3Kdriven 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.

#### 組織特異性

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.

# 関連疾患

Insulin-like growth factor 1 resistance

配列類似性

Belongs to the protein kinase superfamily. Tyr protein kinase family. Insulin receptor subfamily. Contains 4 fibronectin type-III domains.

Contains 1 protein kinase domain.

翻訳後修飾

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.

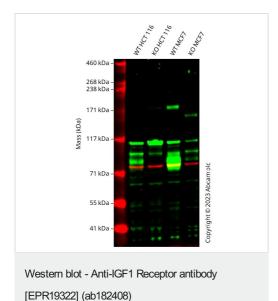
Sumoylated with SUMO1.

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.

### 細胞内局在

Cell membrane.

# 画像



**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

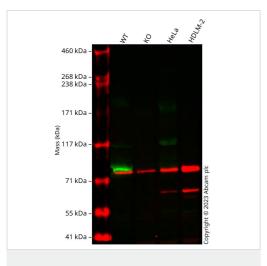
Lane 4: IGF1R knockout MCF7 ab287507 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 156 kDa **Observed band size:** 82 kDa

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] (ab182408)

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

Lane 1: Wild-type MCF7 cell lysate

Lane 2: IGF1R knockout MCF7 cell lysate

Lane 3 : HeLa cell lysate

Lane 4 : HDLM-2 cell lysate

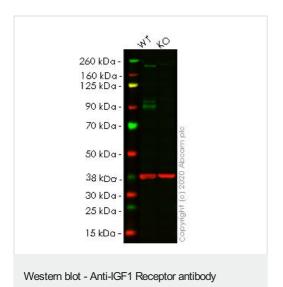
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 156 kDa **Observed band size:** 82 kDa

False colour image of Western blot: Anti-IGF1 Receptor antibody [EPR19322] 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 IGF1 Receptor. A band was observed at 82 kDa (beta chain) in wild-type MCF7 cell lysates with no signal observed at this size in IGF1R knockout cell line. To generate this image, wild-type and IGF1R knockout MCF7 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.



[EPR19322] (ab182408)

All lanes: Anti-IGF1 Receptor antibody [EPR19322] (ab182408) 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.

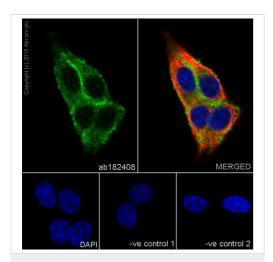
Performed under reducing conditions.

Predicted band size: 156 kDa

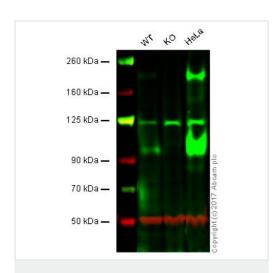
Observed band size: 100 kDa

Lanes 1-2: Merged signal (red and green). Green - ab182408 observed at 100 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab182408 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. ab182408 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 [EPR19322] (ab182408)



Western blot - Anti-IGF1 Receptor antibody [EPR19322] (ab182408)

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 counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) at 1/1000 dilution and Goat Anti-Mouse lgG 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.

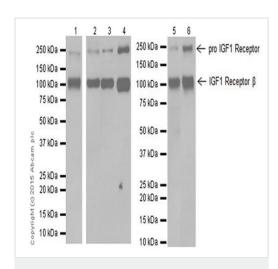
Lane 1: Wild-type HAP1 whole cell lysate (40 µg)

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 <a href="mailto:ab8245">ab8245</a> (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 <a href="mailto:ab216773">ab216773</a> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed <a href="mailto:ab216776">ab216776</a> secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-IGF1 Receptor antibody [EPR19322] (ab182408)

Lanes 1-4: Anti-IGF1 Receptor antibody [EPR19322] (ab182408) at 1/2000 dilution

Lanes 5-6: Anti-IGF1 Receptor antibody [EPR19322] (ab182408) at 1/1000 dilution

Lane 1 : C2C12 (Mouse myoblast cell line) whole cell lysate at 20 µg

**Lane 2 :** HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20  $\mu g$ 

**Lane 3 :** 293 (Human epithelial cell line from embryonic kidney) whole cell lysate at 20  $\mu g$ 

Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate at 20  $\mu g$ 

Lane 5 : C6 (Rat glial tumor cell line) whole cell lysate at 10  $\mu$ g Lane 6 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate at 10  $\mu$ g

# **Secondary**

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

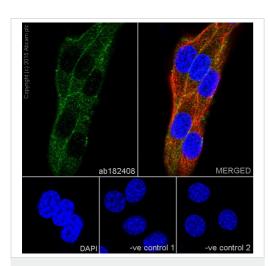
Predicted band size: 156 kDa

Observed band size: 100.200 kDa

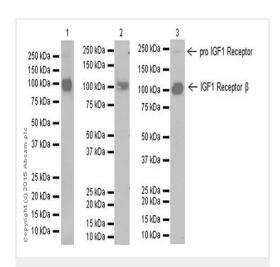
Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 5 seconds; Lane 2-4: 3 minutes; Lane 5/6: 2 seconds.

The expression profile observed is consistent with what has been described in the literature (PMID: 11402025).



Immunocytochemistry/ Immunofluorescence - Anti-IGF1 Receptor antibody [EPR19322] (ab182408)



Western blot - Anti-IGF1 Receptor antibody [EPR19322] (ab182408)

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<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous and cytoplasmic staining on C2C12 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) at 1/1000 dilution and Goat Anti-Mouse lgG 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.

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

**Lanes 2-3**: Anti-IGF1 Receptor antibody [EPR19322] (ab182408) at 1/5000 dilution

Lane 1: Mouse brain lysate

Lane 2: Mouse spleen lysate

Lane 3: Rat brain lysate

Lysates/proteins at 10 µg per lane.

### **Secondary**

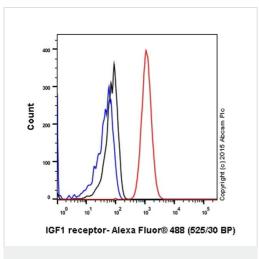
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

**Predicted band size:** 156 kDa **Observed band size:** 100,200 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

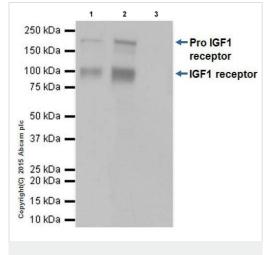
Exposure time: Lane 1: 10 seconds; Lane 2: 3 minutes; Lane 3: 1 minute.

The expression profile observed is consistent with what has been described in the literature (PMID: 11402025).



Flow Cytometry (Intracellular) - Anti-IGF1 Receptor antibody [EPR19322] (ab182408)

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 unlabelled 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.



Immunoprecipitation - Anti-IGF1 Receptor antibody [EPR19322] (ab182408)

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.

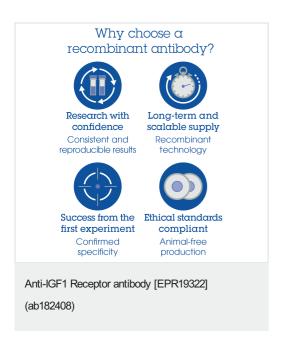
Lane 1: C2C12 whole cell lysate, 10µg (Input).

Lane 2: ab182408 IP in C2C12 whole cell lysate.

Lane 3: Rabbit lgG,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.



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