abcam

Product datasheet

Anti-EGFR antibody [13/EGFR] ab289889

KO 評価済 リコンビナント

画像数 5

製品の概要

製品名	Anti-EGFR antibody [13/EGFR]
製品の詳細	Mouse monoclonal [13/EGFR] to EGFR
由来種	Mouse
アプリケーション	**適用あり:** IP, WB, ICC/IF **適用なし:** Flow Cyt (Intra) or IHC-P
種交差性	**交差種:** 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: Wild type HeLa whole cell lysate, NCI-H1975 whole cell lysate, A431 whole cell lysate. ICC/IF: A431 cells. IP: A431 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**.

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: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 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	13/EGFR
アイソタイプ	IgG1

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ab289889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
IP		1/30.
WB		1/1000. Predicted molecular weight: 134 kDa.
ICC/IF		1/50.

追加情報 Is unsuitable for Flow Cyt (Intra) or IHC-P.

ターゲット情報

機能 Receptor tyrosine kinase binding ligands of the EGF family and activating several signaling cascades to convert extracellular cues into appropriate cellular responses. Known ligands include EGF, TGFA/TGF-alpha, amphiregulin, epigen/EPGN, BTC/betacellulin, epiregulin/EREG and HBEGF/heparin-binding EGF. Ligand binding triggers receptor homo- and/or heterodimerization and autophosphorylation on key cytoplasmic residues. The phosphorylated receptor recruits adapter proteins like GRB2 which in turn activates complex downstream signaling cascades. Activates at least 4 major downstream signaling cascades including the RAS-RAF-MEK-ERK, PI3 kinase-AKT, PLCgamma-PKC and STATs modules. May also activate the NF-kappa-B signaling cascade. Also directly phosphorylates other proteins like RGS16, activating its GTPase activity and probably coupling the EGF receptor signaling to the G protein-coupled receptor signaling. Also phosphorylates MUC1 and increases its interaction with SRC and CTNNB1/beta-catenin.
Isoform 2 may act as an antagonist of EGF action.

組織特異性 Ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.

関連疾患 Lung cancer
Inflammatory skin and bowel disease, neonatal, 2

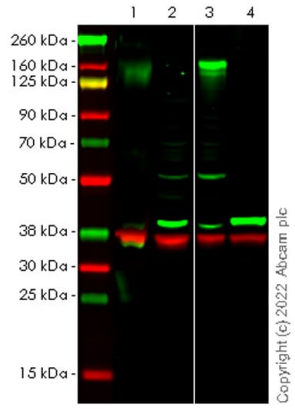
配列類似性 Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 1 protein kinase domain.

翻訳後修飾 Phosphorylation at Ser-695 is partial and occurs only if Thr-693 is phosphorylated. Phosphorylation at Thr-678 and Thr-693 by PRKD1 inhibits EGF-induced MAPK8/JNK1 activation. Dephosphorylation by PTPRJ prevents endocytosis and stabilizes the receptor at the plasma membrane. Autophosphorylation at Tyr-1197 is stimulated by methylation at Arg-1199 and enhances interaction with PTPN6. Autophosphorylation at Tyr-1092 and/or Tyr-1110 recruits STAT3. Dephosphorylated by PTPN1 and PTPN2.
Monoubiquitinated and polyubiquitinated upon EGF stimulation; which does not affect tyrosine kinase activity or signaling capacity but may play a role in lysosomal targeting. Polyubiquitin linkage is mainly through 'Lys-63', but linkage through 'Lys-48', 'Lys-11' and 'Lys-29' also occurs. Deubiquitination by OTUD7B prevents degradation. Ubiquitinated by RNF115 and RNF126. Methylated. Methylation at Arg-1199 by PRMT5 stimulates phosphorylation at Tyr-1197.

細胞内局在 Secreted and Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus membrane. Nucleus membrane. Endosome. Endosome membrane. Nucleus. In response to EGF, translocated from the cell membrane to the nucleus via Golgi and ER. Endocytosed upon activation by ligand. Colocalized with GPER1 in the nucleus of estrogen agonist-induced cancer-

associated fibroblasts (CAF).

画像



Western blot - Anti-EGFR antibody [13/EGFR] (ab289889)

All lanes : Anti-EGFR antibody [13/EGFR] (ab289889) at 1/1000 dilution

Lane 1 : Wild type HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : EGFR knockout HeLa whole cell lysate
Lane 3 : NCI-H1975 (Human adenocarcinoma lung epithelial cell) whole cell lysate
Lane 4 : SW480 (human colorectal adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Mouse IgG H&L (IRDye® 800CW) (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) (**ab216777**) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 134 kDa
Observed band size: 170 kDa

Blocking and diluting buffer: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS.

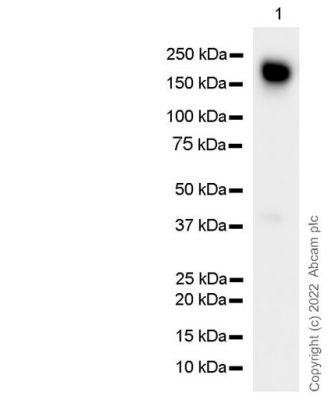
False colour image of Western blot: Anti-EGFR antibody [13/EGFR] (ab289889 staining at 1/1000 dilution, shown in green; Rabbit anti-GAPDH antibody [16891] (**ab181602**) loading control staining at 1/20000 dilution, shown in red.

In Western blot, ab289889 was shown to bind specifically to EGFR. A band was observed at 170 kDa in wild-type HeLa cell lysates with no signal observed at this size in the EGFR knockout cell line. To generate this image, wild-type and EGFR knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a PVDF-FL membrane. Membranes were blocked in Odyssey diluted in equal volume of 0.1 % TBS 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-Mouse IgG H&L (IRDye® 800CW) (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) (**ab216777**) at 1/10000 dilution.

Performed under reducing conditions.

Low expression: SW480(PMID: 19789347)
We observe an unknown band at around 38kDa.



Western blot - Anti-EGFR antibody [13/EGFR] (ab289889)

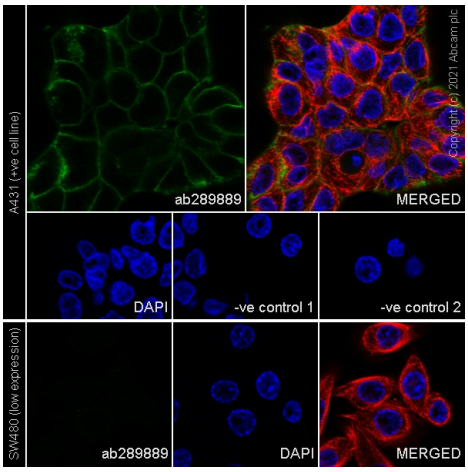
Anti-EGFR antibody [13/EGFR] (ab289889) at 1/1000 dilution + A431 (human epidermoid carcinoma epithelial cell), whole cell lysate at 10 µg

Secondary
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10000 dilution

Predicted band size: 134 kDa
Observed band size: 170 kDa

Exposure time: 15 seconds

Blocking and diluting buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [13/EGFR] (ab289889)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A-431 (Human epidermoid carcinoma cell line) labeling EGFR with ab289889 at 1/50 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) (**ab150117**) preadsorbed secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic and membranous staining in A431 cell line.

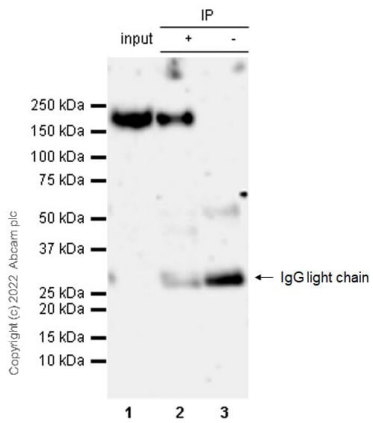
Low expression: SW480 (PMID: 27729020). The nuclear counter stain is DAPI (blue).

Tubulin is counterstained with Anti-alpha Tubulin mouse MAb (**ab179513**) at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150088**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab289889 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150088**) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (**ab179513**) at 1/200 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150117**) secondary antibody at 1/1000 dilution.



Immunoprecipitation - Anti-EGFR antibody [13/EGFR] (ab289889)

EGFR was immunoprecipitated from A431 (human epidermoid carcinoma epithelial cell) whole cell lysate 10 µg with ab289889 at 1/30 diultion. Western Blot was performed from immunoprecipitate using ab289889 at 1/1000 diultion. Mouse IgG for IP (HRP) (**ab131368**) was used as a secondary antibody at 1/5000 dilution.

Lane 1 (Input): A431 (human epidermoid carcinoma epithelial cell) whole cell lysate)

Lane 2 (+): A431 whole cell lysate

Lane 3 (-): Mouse IgG1 monoclonal (**ab18443**) instead of ab289889 in A431 whole cell lysate

Blocking and dilution buffer: 5% NFDM/TBST.

Anti-EGFR antibody [13/EGFR] (ab289889)

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