abcam

Product datasheet

Anti-EGFR (phospho Y1068) antibody [Y38] ab32430

Recombinant RabMAb

37 References 4 Images

Overview

Product name Anti-EGFR (phospho Y1068) antibody [Y38]

Description Rabbit monoclonal [Y38] to EGFR (phospho Y1068)

Host species Rabbit

Specificity This antibody recognises only EGFR phosphorylated on Tyrosine 1068 of the mature human

isoform 1 (corresponding to Y1092 from the precursor form P00533-1/p170).

Suitable for: WB, ICC/IF, IP **Tested applications**

Unsuitable for: Flow Cyt

Reacts with: Human Species reactivity

Predicted to work with: Pig ...

Synthetic peptide within Human EGFR (phospho Y1068). The exact sequence is proprietary. **Immunogen**

Residues surrounding Tyr1068 of mature human EGFR.

Database link: P00533

(Peptide available as ab205609)

Positive control WB: A431 cell lysate treated with EGF. ICC/IF: A431 cell lysate treated with EGF.

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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

Properties

Form Liquid

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long Storage instructions

term. Avoid freeze / thaw cycle.

1

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number Y38 lsotype lgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab32430 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: 135 kDa. A blocking peptide is available for this antibody as ab205609 .
ICC/IF		1/100 - 1/250.
IP		1/30. For unpurified format use at 1/150 dilution.

Application notes Is unsuitable for Flow Cyt.

Target

Function

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/betacatenin.

Isoform 2 may act as an antagonist of EGF action.

Tissue specificity Ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.

Involvement in disease Lung cancer

Inflammatory skin and bowel disease, neonatal, 2

Sequence similaritiesBelongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily.

Contains 1 protein kinase domain.

Post-translational modifications

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

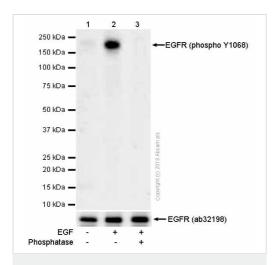
plasma membrane. Autophosphorylation at Tyr-1197 is stimulated by methylation at Arg-1199 and enhances interaction with PTPN6. Autophosphorylation at Tyr-1092 and/or Tyr-1110 recruits STAT3. Dephosphorylated by PTPN1 and PTPN2.

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.

Cellular localization

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).

Images



Western blot - Anti-EGFR (phospho Y1068) antibody

[Y38] (ab32430)

All lanes: Anti-EGFR (phospho Y1068) antibody [Y38] (ab32430) at 1/1000 dilution (Purified)

Lane 1: A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates

Lane 2: A431 (Human epidermoid carcinoma epithelial cell) treated with 100 ng/ml EGF for 30 minutes whole cell lysates

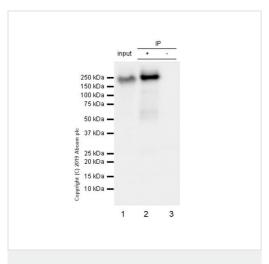
Lane 3: A431 (Human epidermoid carcinoma epithelial cell) treated with 100 ng/ml EGF for 30 minutes. Then the membrane was incubated with phosphatase

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 135 kDa **Observed band size:** 175 kDa



Immunoprecipitation - Anti-EGFR (phospho Y1068) antibody [Y38] (ab32430)

Ab52894
Anti-EGFR (phospho Y1088)
Ab52894
Anti-EGFR
Ab32430
Ab52894
Ab

Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho Y1068) antibody [Y38] (ab32430)

ab32430 (purified) at 1/30 dilution (2ug) immunoprecipitating EGFR in A431 treated with 200 ng/ml EGF for 15 minutes whole cell lysate 10ug . A431 (Human epidermoid carcinoma epithelial cell) treated with 200 ng/ml EGF for 15 minutes whole cell lysate 10ug

Lane 2 (+): ab32430 & A431 treated with 200 ng/ml EGF for 15 minutes whole cell lysate 10ug

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32430 in A431 treated with 200 ng/ml EGF for 15 minutes whole cell lysate 10ug

For western blotting, VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/1000 dilution.

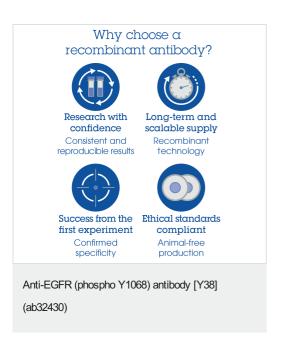
Blocking and diluting buffer: 5% NFDM/TBST.

Immunocytochemistry/Immunofluorescence analysis of untreated, EGF treated and EGF + LP treated A431 cells labelling EGFR (phospho Y1068) with purified ab32430 (left) and EGFR with **ab52894** (right) both at a dilution of 1/1000.

Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor 594-conjugated goat anti-mouse IgG (1/1000) were also used.

The image shows increased membrane staining after EGF (100ng/ml, 30min) treatment on A431 cells. The LP treatment decreased the increased membrane staining caused by EGF.

<u>ab52894</u> was used as a Pan control for ab32430. The results showed membrane and cytoplasmic staining on untreated, EGF and EGF + LP treated A431 cells.



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