abcam

Product datasheet

Anti-EGFR (phospho S1046 + S1047) antibody [EP2259Y] ab76300

Recombinant RabMAb

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Overview

Product name Anti-EGFR (phospho S1046 + S1047) antibody [EP2259Y]

Description Rabbit monoclonal [EP2259Y] to EGFR (phospho S1046 + S1047)

Host species Rabbit

Specificity Recognises EGFR phosphorylated on Serine 1046 and Serine 1047 of the mature human

isoform 1 (corresponding to S1070 and S1071 from the precursor form P00533-1/p170)

Tested applications Suitable for: WB, IP, ICC/IF, Dot blot

Unsuitable for: Flow Cyt or IHC-P

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: A431 cell lysates treated with EGF. ICC/IF: A431 cells treated with EGF. IP: A431 treated

with EGF whole cell lysate. Dot Blot: EGFR (pS1046/pS1047) phospho peptide.

General notesOur 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

Storage instructions Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer pH: 7.20

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

culture supernatant

Purity Protein A purified

Clonality Monoclonal
Clone number EP2259Y

1

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab76300 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)	1/500000 - 1/1e+006. Detects a band of approximately 150 kDa (predicted molecular weight: 134 kDa).
IP		1/40.
ICC/IF		1/100 - 1/250.
Dot blot		1/1000.

Application notes

Is unsuitable for Flow Cyt or IHC-P.

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/beta-catenin.

lsoform 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 similarities

Belongs 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.

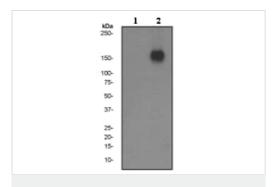
Monoubiquitinated and polyubiquitinated upon EGF stimulation; which does not affect tyrosine kinase activity or signaling capacity but may play a role in lysosomal targeting. Polyubiquitin linkage is mainly through 'Lys-63', but linkage through 'Lys-48', 'Lys-11' and 'Lys-29' also occurs.

Cellular localization

Deubiquitination by OTUD7B prevents degradation. Ubiquitinated by RNF115 and RNF126. Methylated. Methylation at Arg-1199 by PRMT5 stimulates phosphorylation at Tyr-1197.

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 S1046 + S1047) antibody [EP2259Y] (ab76300)

All lanes : Anti-EGFR (phospho S1046 + S1047) antibody [EP2259Y] (ab76300) at 1/1000000 dilution

Lane 1: A431 cell lysate

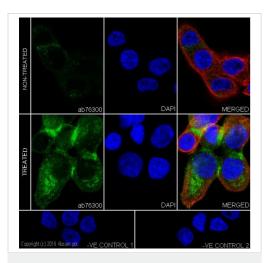
Lane 2: A431 cell lysate treated with EGF

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: HRP labelled goat anti-rabbit at 1/2000 dilution

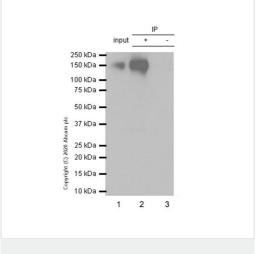
Predicted band size: 134 kDa **Observed band size:** 180 kDa



Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho S1046 + S1047) antibody [EP2259Y] (ab76300) Immunocytochemistry/Immunofluorescence analysis of untreated and EGF (100ng/mL for 10 minutes) treated A431 cells labelling EGFR (phospho S1046 + S1047) with ab76300 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor[®] 488-conjugated goat antirabbit lgG (1/1000) was used as the secondary antibody. The cells were co-stained with ab195889, an Alexa Fluor[®] 594-conjugated mouse anti-tubulin (1/200). Nuclei counterstained with DAPI (blue).

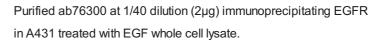
Control 1: untreated A431 cells incubated with PBS instead of primary antibody followed by incubation with ab150077 Alexa Fluor[®] 488 goat anti-rabbit lgG.

Control 2: EGF treated A431 cells incubated with PBS instead of primary antibody followed by incubation with ab150077 Alexa Fluor[®] 488 goat anti-rabbit lgG.



Immunoprecipitation - Anti-EGFR (phospho S1046 +

S1047) antibody [EP2259Y] (ab76300)



Lane 1 (input): A431 (Human epidermoid carcinoma epithelial cell) treated with EGF whole cell lysate 10µg

Lane 2 (+): ab76300 + A431 treated with EGF whole cell lysate.

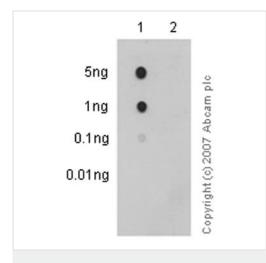
Lane 3 (-): Rabbit monoclonal lgG (ab172730) instead of ab76300 in A431 treated with EGF whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (ab131366) (1/1000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 180 kDa



Dot Blot - Anti-EGFR (phospho S1046 + S1047) antibody [EP2259Y] (ab76300)

Primary antibody: ab76300 at a dilution of 1/1000.

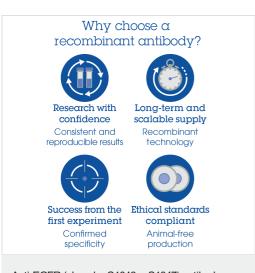
Secondary antibody: Peroxidase conjugated-goat anti-rabbit lgG, (H+L) at a dilution of 1/2500.

Blocking and dilution buffer: 5% NFDM/TBST

Lane 1: EGFR (pS1046/pS1047) phospho peptide.

Lane 2: EGFR non-phospho peptide.

Exposure time: 3 minutes.



Anti-EGFR (phospho S1046 + S1047) antibody [EP2259Y] (ab76300)

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