abcam

Product datasheet

Anti-EGFR (phospho S1046 + S1047) antibody [EP2259Y] - BSA and Azide free ab256225

Recombinant RabMAb

4 Images

Overview

Product name	Anti-EGFR (phospho S1046 + S1047) antibody [EP2259Y] - BSA and Azide free
Description	Rabbit monoclonal [EP2259Y] to EGFR (phospho S1046 + S1047) - BSA and Azide free
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	ICC/IF: A431 cells treated with EGF. IP: A431 treated with EGF whole cell lysate. Dot Blot: EGFR (pS1046/pS1047) phospho peptide.
General notes	ab256225 is the carrier-free version of **ab76300**. 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. 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
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
Clonality	Monoclonal
Clone number	EP2259Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab256225 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 150 kDa (predicted molecular weight: 134 kDa).
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
Dot blot		Use at an assay dependent concentration.

Application notes Is unsuitable for Flow Cyt or IHC-P.

Target

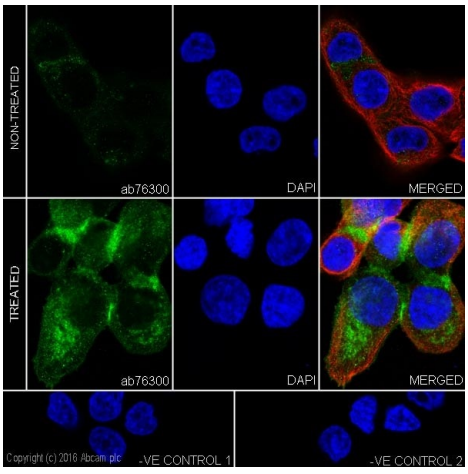
Function Receptor tyrosine kinase binding ligands of the EGF family and activating several signaling cascades to convert extracellular cues into appropriate cellular responses. Known ligands include EGF, TGFA/TGF-alpha, amphiregulin, epigen/EPGN, BTC/betacellulin, epiregulin/EREG and HBEGF/heparin-binding 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.
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 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. 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

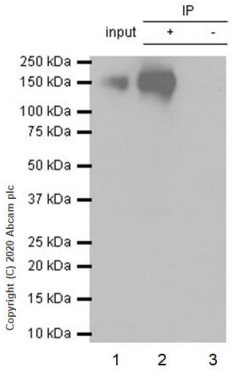


Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho S1046 + S1047) antibody [EP2259Y] - BSA and Azide free (ab256225)

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 anti-rabbit IgG (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).

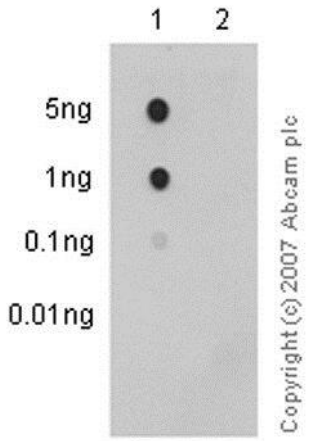
Control 1: untreated A431 cells incubated with PBS instead of primary antibody followed by incubation with **ab150077** Alexa Fluor® 488 goat anti-rabbit IgG.
Control 2: EGF treated A431 cells incubated with PBS instead of primary antibody followed by incubation with **ab150077** Alexa Fluor® 488 goat anti-rabbit IgG.

This data was developed using the same antibody clone in a different buffer formulation containing Tris Glycine, BSA, glycerol, and sodium azide (**ab76300**).



Immunoprecipitation - Anti-EGFR (phospho S1046 + S1047) antibody [EP2259Y] - BSA and Azide free (ab256225)

This data was developed using **ab76300**, the same antibody clone in a different buffer formulation.
Purified **ab76300** at 1/40 dilution (2µg) immunoprecipitating EGFR in A431 treated with EGF whole cell lysate.
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 IgG (**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] - BSA and Azide free (ab256225)

This data was developed using the same antibody clone in a different buffer formulation (**ab76300**).

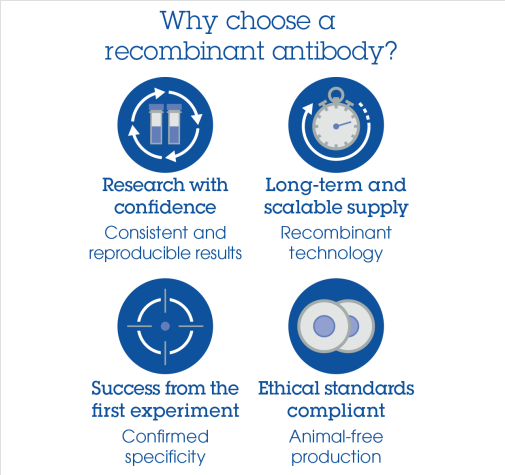
Primary antibody: **ab76300** at a dilution of 1/1000.

Secondary antibody: Peroxidase conjugated-goat anti-rabbit IgG, (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.



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