abcam

Product datasheet

Anti-EGFR (phospho Y1086) antibody ab5650

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Overview

Product name	Anti-EGFR (phospho Y1086) antibody
Description	Rabbit polyclonal to EGFR (phospho Y1086)
Host species	Rabbit
Tested applications	**Suitable for:** ICC, WB
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide corresponding to Human EGFR (phospho Y1086).
Positive control	WB: A-431 cells, A549 cells. ICC: A-431 cells.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.3 Constituent: PBS
Purity	Immunogen affinity purified
Purification notes	The antibody has been negatively preadsorbed using (i) a non phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated epidermal growth factor receptor (EGFR), and (ii) a generic tyrosine phosphorylated peptide to remove antibody that is reactive with phosphotyrosine, irrespective of the sequence. The final product is generated by affinity chromatography using an EGFR-derived peptide that is phosphorylated at tyrosine 1086.
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab5650 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
ICC		1/100.
WB	★★★★☆ (1)	Use a concentration of 0.1 - 1 µg/ml. Detects a band of approximately 185 kDa.

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

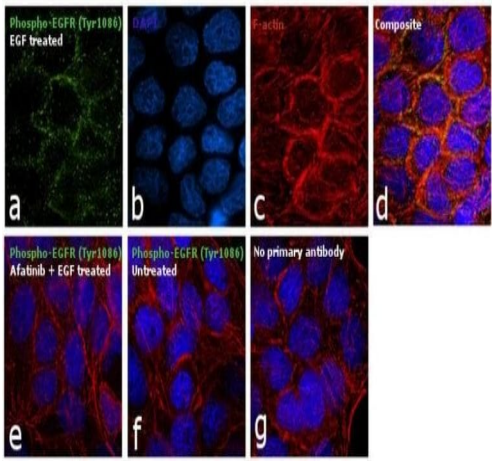
Sequence similarities Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 1 protein kinase domain.

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-

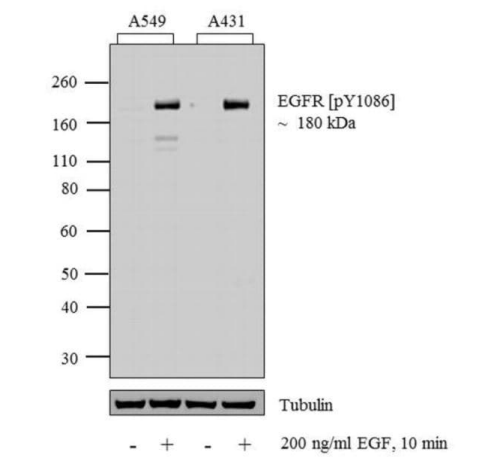
associated fibroblasts (CAF).

Images



Immunocytochemistry - Anti-EGFR (phospho Y1086) antibody (ab5650)

A-431 cells stained for EGFR (green) using ab5650 at 1/100 dilution in ICC/IF. Followed by Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate at 1/2000 dilution for 45 minutes at room temperature (Panel a). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI. F-actin (Panel c: red) was stained with Rhodamine Phalloidin at 1/300 dilution. Panel d represents the merged image showing membranous localization. Panel e represents cells treated with antagonist, Afatinib (1uM for 6hrs) followed by EGF (200 ng/mL for 10 minutes), showing no signal. Panel f shows untreated cells with no signal. Panel g represents control cells with no primary antibody to assess background.



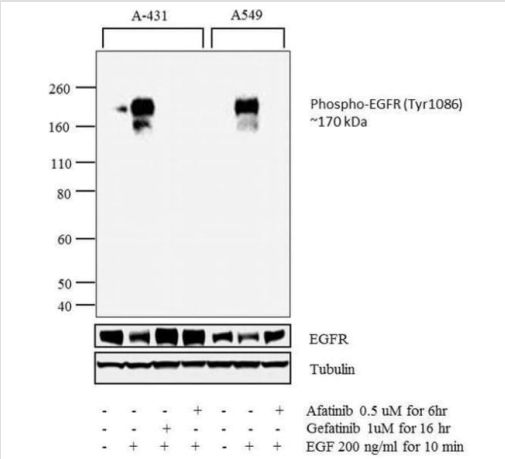
Western blot - Anti-EGFR (phospho Y1086) antibody (ab5650)

All lanes : Anti-EGFR (phospho Y1086) antibody (ab5650) at 1/1000 dilution

Lane 1 : A549 cell lysate
Lane 2 : A549 cell lysate treated for 10 minutes with 200 ng/mL of EGF
Lane 3 : A431 cell lysate
Lane 4 : A431 cell lysate treated for 10 minutes with 200 ng/mL of EGF

Lysates/proteins at 30 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG - HRP Secondary Antibody at 1/5000 dilution



Western blot - Anti-EGFR (phospho Y1086) antibody (ab5650)

All lanes : Anti-EGFR (phospho Y1086) antibody (ab5650) at 1/1000 dilution

Lane 1 : A-431 extracts
Lane 2 : A-431 treated with EGF (200 ng/mL for 10 minutes)
Lane 3 : A-431 treated with Gefitinib followed by EGF (1uM for 16 hours, 200 ng/mL for 10 minutes)
Lane 4 : A-431 treated with Afatinib followed by EGF (0.5 uM for 6 hours, 200 ng/mL for 10 minutes)
Lane 5 : A549 extracts
Lane 6 : A549 treated with EGF (200 ng/mL for 10 minutes)
Lane 7 : A549 treated with Afatinib followed by EGF (0.5 uM for 6 hours, 200 ng/mL for 10 minutes)

Lysates/proteins at 30 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate at 1/4000 dilution

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