abcam

Product datasheet

Anti-EGFR (phospho Y992) antibody ab5638

2 References 4 Images

Overview

Product name Anti-EGFR (phospho Y992) antibody

Description Rabbit polyclonal to EGFR (phospho Y992)

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC

Species reactivity Reacts with: Human

Immunogen Synthetic peptide corresponding to Human EGFR (phospho Y992).

Positive control A431 cells stimulated with EGF; human lung adenocarcinoma, human hepatocellular carcinoma

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. Avoid freeze / thaw cycles.

Storage buffer pH: 7.30

Preservative: 0.05% Sodium azide

Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.1% BSA

PBS is without Mg2+ and Ca2+ and BSA is lgG and protease free.

Purity Immunogen affinity purified

Purification notesThe 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 922.

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Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab5638 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. Detects a band of approximately 170 kDa.
IHC-P		1/10 - 1/1000.
ICC		1/100 - 1/500.

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

Contains 1 protein kinase domain.

Post-translational modifications

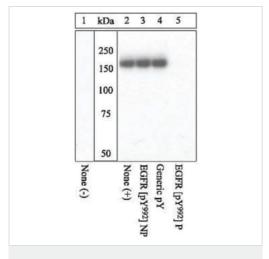
Phosphorylation at Ser-695 is partial and occurs only if Thr-693 is phosphorylated. Phosphorylation at Thr-678 and Thr-693 by PRKD1 inhibits EGF-induced MAPK8/JNK1 activation. Dephosphorylation by PTPRJ prevents endocytosis and stabilizes the receptor at the plasma membrane. Autophosphorylation at Tyr-1197 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

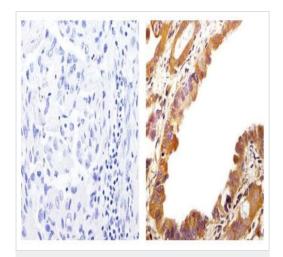


Western blot - Anti-EGFR (phospho Y992) antibody (ab5638)

All lanes : Anti-EGFR (phospho Y992) antibody (ab5638) at 1/1000 dilution

Lane 1: A431 cells unstimulated

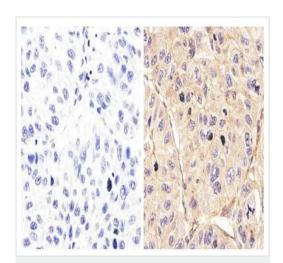
Lanes 2-5: A431 cells stimulated with 200 ng/mL EGF for 15 minutes



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EGFR (phospho Y992) antibody (ab5638)

Immunohistochemistry analysis of EGFR (pY992) with ab5638 at 1/20 dilution showing staining in the cytoplasm and membrane of paraffin-embedded human lung adenocarcinoma (right) compared to a negative control without primary antibody (left).

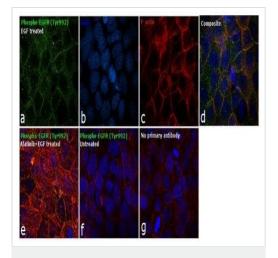
Antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EGFR (phospho Y992) antibody (ab5638)

Immunohistochemistry analysis of EGFR (pY992) with ab5638 at 1/20 dilution showing staining in the cytoplasm and membrane of paraffin-embedded human hepatocellular carcinoma (right) compared to a negative control without primary antibody (left).

Antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min.



Immunocytochemistry - Anti-EGFR (phospho Y992) antibody (ab5638)

Immunocytochemical analysis of EGFR [pY992] was performed using A-431 cells treated with 200 ng/mL of EGF for 10 minutes. The cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton™ X-100, and blocked with 1% BSA. The cells were labeled with ab5638 at 1/250 dilution and incubated overnight at 4°C followed by a Goat anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor® 488 conjugate at a dilution of 1/2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) DAPI (Panel c: red) F-actin was stained with Rhodamine Phalloidin at 1/300. Panel d represents the merged image showing membranous localization. Panel e represents cells treated with antagonist, Afatinib (1µM for 6hrs) followed by EGF (200 ng/mL for 10 minutes), showing reduced Phospho-EGFR staining. Panel f shows untreated cells with no signal. Panel g represents control cells with no primary antibody to assess background.

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