# abcam

## Product datasheet

## Anti-EGFR (phospho Y1173) antibody ab5652

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#### Overview

Product name Anti-EGFR (phospho Y1173) antibody

**Description** Rabbit polyclonal to EGFR (phospho Y1173)

Host species Rabbit

Tested applications

Suitable for: WB, ICC, IHC-P

Species reactivity

Reacts with: Mouse, Human

**Immunogen** Synthetic peptide corresponding to Human EGFR (phospho Y1173).

**Positive control** WB: NIH/3T3 cells. IHC-P: Human hepatocellular carcinoma, human lung adenocarcinoma. ICC:

HeLa 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. Avoid freeze / thaw cycles.

Storage buffer pH: 7.30

Preservative: 0.05% Sodium azide

Constituents: PBS, 50% Glycerol, 0.1% BSA

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 1173.

**Clonality** Polyclonal

1

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab5652 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	*** <u>*</u> (1)	1/1000. Detects a band of approximately 185 kDa.
ICC		1/100 - 1/500.
IHC-P		1/10 - 1/100.

#### **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.

**Tissue specificity** 

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

Isoform 2 may act as an antagonist of EGF action.

Inflammatory skin and bowel disease, neonatal, 2

Involvement in disease

Sequence similarities

Lung cancer

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.

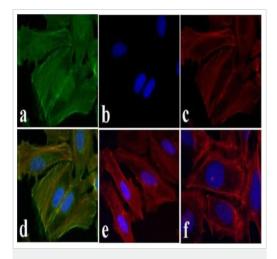
Monoubiquitinated and polyubiquitinated upon EGF stimulation; which does not affect tyrosine kinase activity or signaling capacity but may play a role in lysosomal targeting. Polyubiquitin linkage is mainly through 'Lys-63', but linkage through 'Lys-48', 'Lys-11' and 'Lys-29' also occurs. Deubiquitination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

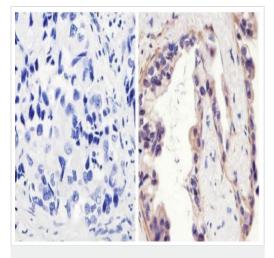
translocated from the cell membrane to the nucleus via Golgi and ER. Endocytosed upon activation by ligand. Colocalized with GPER1 in the nucleus of estrogen agonist-induced cancer-associated fibroblasts (CAF).

## **Images**



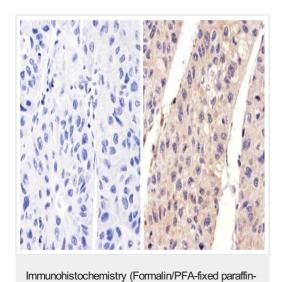
Immunocytochemistry - Anti-EGFR (phospho Y1173) antibody (ab5652)

HeLa cells stained for EGFR (green) using ab5652 at 2 µg/mL in ICC/IF. Followed by Alexa Fluor 488 Goat Anti-Rabbit IgG Secondary Antibody at 1/400 dilution for 30 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 Alexa Fluor 594 Phalloidin. Panel d is a merged image showing cytoplasmic localization of EGFR (pY1173). Panel e shows untreated cells. Panel f shows no primary antibody control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EGFR (phospho Y1173) antibody (ab5652)

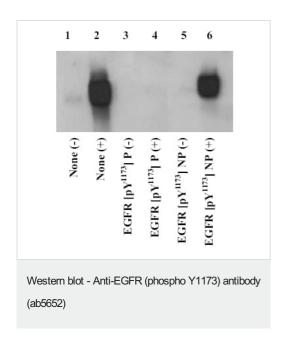
Paraffin embedded human lung adenocarcinoma tissue stained for EGFR using ab5652 at 1/50 dilution in immunohistochemical analysis.



embedded sections) - Anti-EGFR (phospho Y1173)

antibody (ab5652)

Paraffin embedded human hepatocellular carcinoma tissue stained for EGFR using ab5652 at 1/20 dilution in immunohistochemical analysis.



Cell extracts prepared from NIH3T3 cells expressing EGFR were starved for 30 hours, then stimulated for 10 minutes with 30 ng/mL EGF (+), or left unstimulated (-), then resolved by SDS-PAGE on a 6% Tris-glycine gel, and transferred to nitrocellulose. Membranes were incubated with 0.50 µg/mL ab5652 antibody, following prior incubation in the absence (lanes 1& 2), or presence of the peptide immunogen (lanes 3 & 4), or the nonphosphopeptide corresponding to the EGFR phosphopeptide (lanes 5 & 6). After washing, membranes were incubated with goat F(ab')2 anti-rabbit lgG alkaline phosphatase and bands were detected using the Tropix WesternStar detection method. The data show that only the phosphopeptide corresponding to this site blocks the antibody signal, demonstrating the specificity of the ab5652 antibody for this phosphorylated residue. Cell extracts prepared from NIH3T3 cells expressing EGFR were starved for 30 hours, then stimulate

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