abcam

Product datasheet

# Anti-EGFR antibody [EGFR1] ab30

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## Overview

| | |
|---|---|
| **Product name** | Anti-EGFR antibody [EGFR1] |
| **Description** | Mouse monoclonal [EGFR1] to EGFR |
| **Host species** | Mouse |
| **Specificity** | Recognises the external EGF-binding domain of the EGFR transmembrane glycoprotein. No effect on tyrosine kinase activity of EGFR. |
| **Tested applications** | **Suitable for:** IHC-Fr, ICC/IF, Flow Cyt<br>**Unsuitable for:** ELISA or WB |
| **Species reactivity** | **Reacts with:** Human<br>**Predicted to work with:** Mouse, Horse |
| **Immunogen** | Tissue, cells or virus. This information is proprietary to Abcam and/or its suppliers. |
| **Epitope** | Extracellular epitope mapped between aa 6-273 of human EGFR. |
| **Positive control** | IHC-Fr: Frozen normal human placenta ICC/IF: A431 cells. Flow Cyt: A431 cells |
| **General notes** | This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**.<br><br>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.<br><br>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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle. |
| **Storage buffer** | Preservative: 0.02% Sodium azide<br>Constituents: PBS, 6.97% L-Arginine |
| **Purity** | Protein G purified |

| | |
|---|---|
| **Clonality** | Monoclonal |
| **Clone number** | EGFR1 |
| **Myeloma** | P3-NS1/1-Ag4-1 |
| **Isotype** | IgG2b |
| **Light chain type** | kappa |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab30 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 |
|---|---|---|
| **IHC-Fr** | | Use a concentration of 10 µg/ml. |
| **ICC/IF** | ★★★☆☆ (1) | Use a concentration of 1 µg/ml. |
| **Flow Cyt** | | Use a concentration of 1 µg/ml.<br>**ab170192** - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody. |

**Application notes** Is unsuitable for ELISA or WB.

## 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.

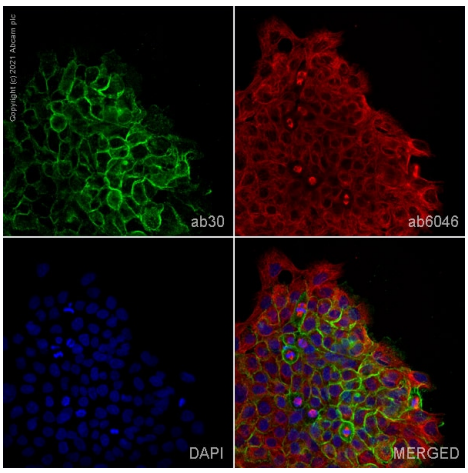
**Post-translational modifications** Phosphorylation at Ser-695 is partial and occurs only if Thr-693 is phosphorylated. Phosphorylation at Thr-678 and Thr-693 by PRKD1 inhibits EGF-induced MAPK8/JNK1 activation. Dephosphorylation by PTPRJ prevents endocytosis and stabilizes the receptor at the plasma 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).

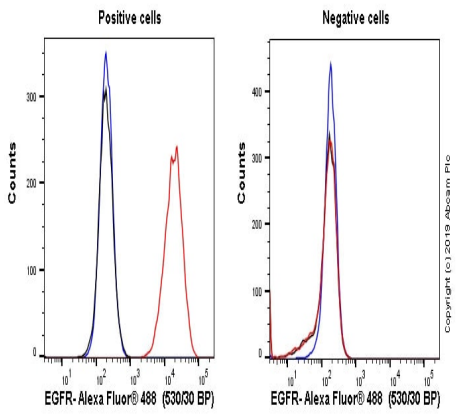
## Images




Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [EGFR1] (ab30)

ab30 staining EGFR in A431 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab30 at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

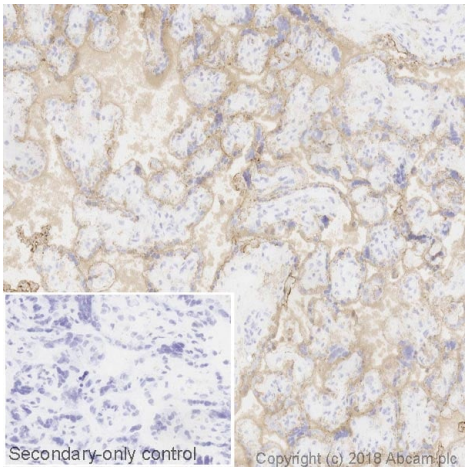



Flow Cytometry - Anti-EGFR antibody [EGFR1] (ab30)

Overlay histograms showing left positive A431 cells and right negative Jurkat cells stained with ab30 (red line). The cells were incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab30, $1x10^6$ cells in 100ul at 1ug/ml) for 30 min on ice. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) preabsorbed (**ab150117**) at 1/2000 dilution for 30 min on ice.

Isotype control antibody (black line) was mouse IgG2bκ (**ab170192**) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.




Immunohistochemistry (Frozen sections) - Anti-EGFR antibody [EGFR1] (ab30)

IHC image of EGFR staining in a section of frozen normal human placenta, fixed in 10% paraformaldehyde (10 min). Staining was performed on a Leica BOND™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab30, 10ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



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