abcam

Product datasheet

Anti-EGFR antibody [EMab-134] ab264540

Recombinant

3 References 6 Images

Overview

Product name Anti-EGFR antibody [EMab-134]

Description Mouse monoclonal [EMab-134] to EGFR

Host species Mouse

Tested applications Suitable for: IHC-P, Flow Cyt, WB

Species reactivity Reacts with: Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: A431 and MDA-MB-468 whole cell lysates. IHC-P: Human oral carcinoma and lung

carcinoma tissue; Human skin tissue. 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.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

Improved sensitivity and specificityLong-term security of supplyAnimal-free production

For more information see here.

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 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)

Purity Protein A purified

Clonality Monoclonal
Clone number EMab-134

lsotype lgG1

1

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab264540 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-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		1/100.
WB		1/1000. Detects a band of approximately 130, 180 kDa (predicted molecular weight: 134 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

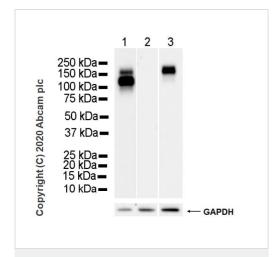
Phosphorylation at Ser-695 is partial and occurs only if Thr-693 is phosphorylated. Phosphorylation at Thr-678 and Thr-693 by PRKD1 inhibits EGF-induced MAPK8/JNK1 activation. Dephosphorylation by PTPRJ prevents endocytosis and stabilizes the receptor at the plasma membrane. Autophosphorylation at Tyr-1197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



Western blot - Anti-EGFR antibody [EMab-134] (ab264540)

All lanes : Anti-EGFR antibody [EMab-134] (ab264540) at 1/1000 dilution

Lane 1 : A431 (human epidermoid carcinoma epithelial cell), whole cell lysate

Lane 2 : SW620 (human colorectal adenocarcinoma epithelial cell), whole cell lysate

Lane 3 : MDA-MB-468 (human breast adenocarcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10000 dilution

Predicted band size: 134 kDa **Observed band size:** 130,180 kDa

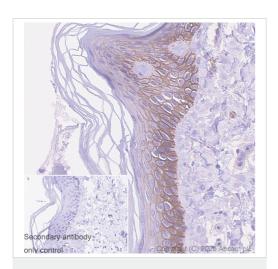
Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lane 1, 2: 15 secs; Lane 3: 8 secs.

The molecular weight observed is in consistent with the literature

(PMID: 9090976).

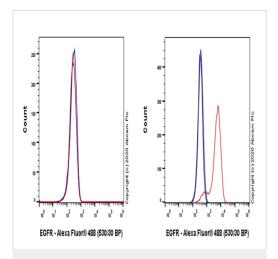
Negative control: SW620 (PMID: 19789347).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EGFR antibody [EMab-134] (ab264540)

Immunohistochemical analysis of paraffin-embedded human skin tissue labeling EGFR with ab264540 at 1/100 dilution followed by a ready to use secondary. Membranous staining on human skin is observed. The section was incubated with ab264540 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is ready to use.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

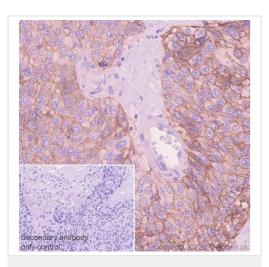


Flow Cytometry - Anti-EGFR antibody [EMab-134] (ab264540)

Flow cytometric analysis of SW620 (human colorectal adenocarcinoma epithelial cell, Left panel), A431 (human epidermoid carcinoma epithelial cell, Right panel) cells labelling EGFR with ab264540 at 1/100 dilution (1µg) (Red) compared with a mouse monoclonal lgG (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti mouse lgG (Alexa Fluor® 488, ab150113) at 1/2000 dilution was used as the secondary antibody.

Negative control: SW620.

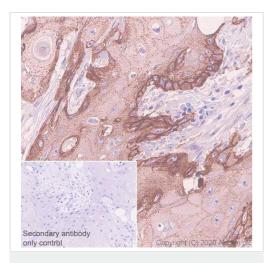
Gated on viable cells.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EGFR antibody [EMab-134] (ab264540)

Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue labeling EGFR with ab264540 at 1/100 dilution followed by a ready to use secondary. Membranous staining on human lung carcinoma tissue is observed. The section was incubated with ab264540 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND®RX instrument. Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is ready to use.

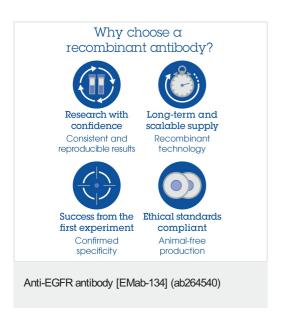
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-EGFR antibody [EMab-134] (ab264540)

Immunohistochemical analysis of paraffin-embedded human oral carcinoma tissue labeling EGFR with ab264540 at 1/100 dilution followed by a ready to use secondary. Membranous staining on human oral carcinoma tissue is observed. The section was incubated with ab264540 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is ready to use.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



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