abcam

Product datasheet

Anti-EGFR antibody [E234] ab32198

敲除验证 重组 RabMAb

3 Abreviews 4 References 8 图像

概述

产品名称	Anti-EGFR抗体[E234]
描述	兔单克隆抗体[E234] to EGFR
宿主	Rabbit
特异性	b32198 detects Epidermal growth factor receptor (EGFR). It does not cross react with other ERBB family members. This product yielded a strong signal in western blot using A431 (human squamous carcinoma) lysate which naturally overexpresses the EGFR protein. Western blot conditions may need to be optimised for cell lines and tissues that express lower levels of endogenous EGFR.
经测试应用	**适用于:** Flow Cyt (Intra), WB, ICC/IF, IHC-P
种属反应性	**与反应:** Human **预测可用于:** Cow, Pig
免疫原	Synthetic peptide corresponding to Human EGFR aa 1-100 (N terminal). Database link: **P00533**
阳性对照	WB: HeLa, A549 and A431 cell lysates IHC-P: FFPE human skin tissue sections, human pancreatic carcinoma tissue. ICC/IF: A431 cells. Flow Cyt (intra): A431 cells.
常规说明	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

性能

形式	Liquid

存放说明	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.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	E234
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab32198于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/200. For unpurified use at 1/50. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/1000. Detects a band of approximately 134 kDa (predicted molecular weight: 180 kDa). **For unpurified use at 1/500.** This product yielded a strong signal in western blot using A431 (human squamous carcinoma) lysate which naturally overexpresses the EGFR protein. Western blot conditions may need to be optimised for cell lines and tissues that express lower
ICC/IF	★★★★★ (1)	1/100.
IHC-P	★★★★☆ (1)	1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

靶标

功能 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.

组织特异性 Ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.

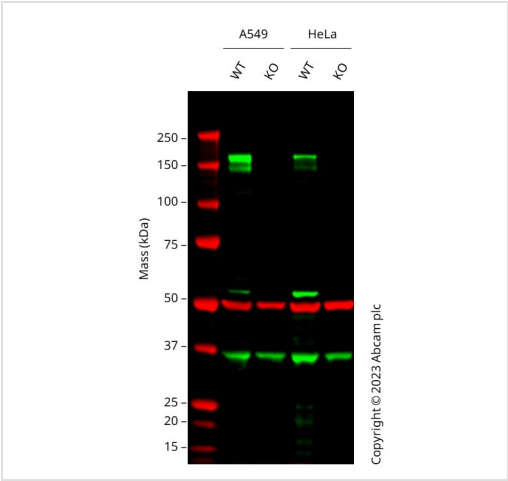
疾病相关 Lung cancer
Inflammatory skin and bowel disease, neonatal, 2

序列相似性 Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 1 protein kinase domain.

翻译后修饰 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.

细胞定位 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).

图片



Western blot - Anti-EGFR antibody [E234] (ab32198)

All lanes : Anti-EGFR antibody [E234] (ab32198) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : EGFR knockout A549 cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : EGFR knockout HeLa cell lysate

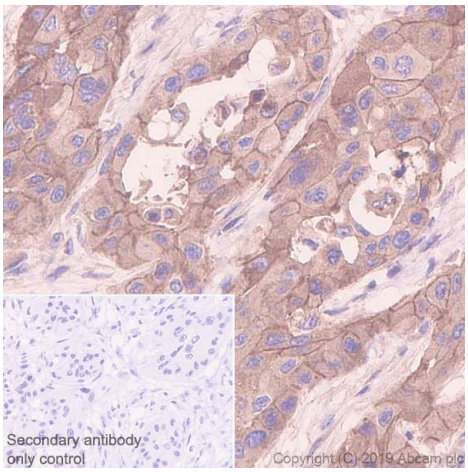
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 180 kDa
Observed band size: 160 kDa

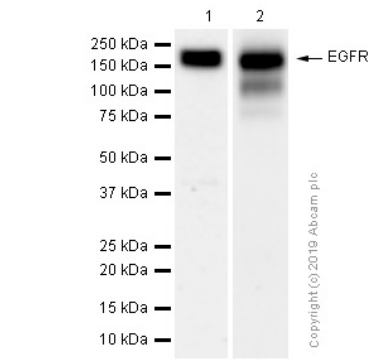
Western blot: Anti-EGFR antibody [E234] (ab32198) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A]

(**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32198 was shown to bind specifically to EGFR. A band was observed at 160 kDa in wild-type cell lysates with no signal observed at this size in EGFR knockout cell lines. To generate this image, wild-type and EGFR knockout A549 (**ab286394**) and HeLa (**ab255385**) cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% milk in TBS-0.1% Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [E234] (ab32198)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human pancreatic carcinoma tissue sections labeling EGFR with purified ab32198 at 1/100 dilution (2.22 µg/mL). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-EGFR antibody [E234] (ab32198)

All lanes : Anti-EGFR antibody [E234] (ab32198) at 1/1000 dilution (Purified)

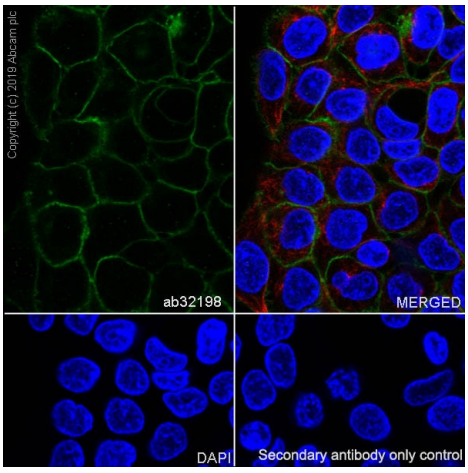
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

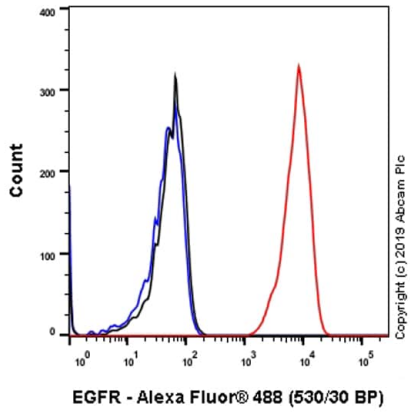
Predicted band size: 180 kDa
Observed band size: 150 kDa

Blocking/Diluting Buffer and concentration: 5% NFDM/TBST



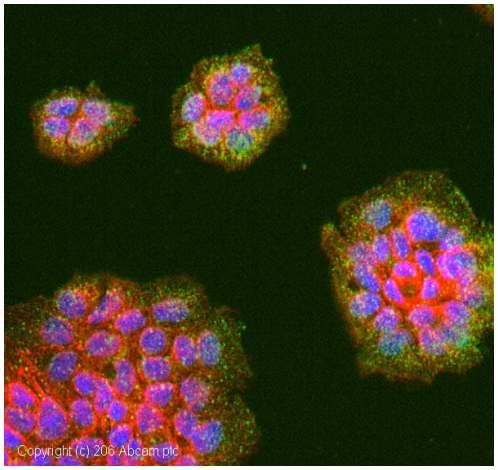
Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [E234] (ab32198)

Immunocytochemistry/ Immunofluorescence analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling EGFR with purified ab32198 at 1/100 dilution (2.2 µg/mL). Cells were fixed in 100% Methanol. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



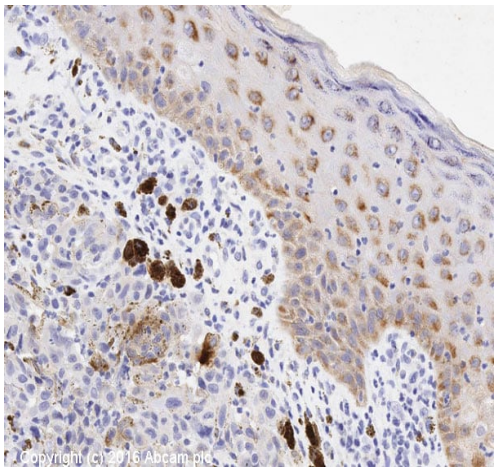
Flow Cytometry (Intracellular) - Anti-EGFR antibody [E234] (ab32198)

Intracellular Flow Cytometry analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling EGFR with purified ab32198 at 1/200 dilution (10µg/mL) (Red). Cells were fixed with 80% Methanol and permeabilised with 0.1% Tween-20. A Goat anti rabbit IgG (Alexa Fluorr® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [E234] (ab32198)

ab32198 (unpurified) stained A431 cells. The cells were 100% methanol fixed for 10 minutes at -20°C and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1hour at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab32198 at 1in100 dilution) overnight at +4°C. The secondary antibody (pseudo-colored green) was **Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081)** used at a 1/1000 dilution for 1hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [E234] (ab32198)

IHC image of EGFR staining in human skin formalin fixed paraffin embedded tissue section*, 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 20 mins. The section was then incubated with ab32198 (unpurified) at 1/100 dilution 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.

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.

**Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre*

Anti-EGFR antibody [E234] (ab32198)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery

- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.cn/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors