abcam

Product datasheet

Anti-EGFR antibody [EP38Y] - Low endotoxin, Azide free ab174481

敲除验证 重组 RabMAb

9 图像

概述

产品名称	Anti-EGFR抗体[EP38Y] - Low endotoxin, Azide free
描述	兔单克隆抗体[EP38Y] to EGFR - Low endotoxin, Azide free
宿主	Rabbit
特异性	The immunogen for this product is a synthetic phospho-peptide corresponding to residues surrounding Tyr1068 of human EGFR. After screening, clone EP38Y was found to recognize total EGFR and is not specific to phosphorylated-Tyr1068 EGFR. 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. The mouse and rat recommendation is based on the WB results. This antibody may not be suitable for IHC with mouse or rat samples.
经测试应用	**适用于:** Indirect ELISA, Flow Cyt (Intra), IHC-P, WB, ICC/IF, IP
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: A431, HeLa, Caco-2 and MDA-MB-468 cell lysates. Wild-type HCT 116 cell lysate. IHC-P: Human cervical carcinoma tissue/. IP: HeLa whole cell lysate. Flow Cyt: A431cells. ICC: A431 cells.
常规说明	ab174481 is the carrier-free version of **ab52894**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C. Do Not Freeze.
存储溶液	Constituent: PBS
无载体	**是**
纯度	Protein A purified
克隆	单克隆
克隆编号	EP38Y
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab174481于以下的经测试应用**

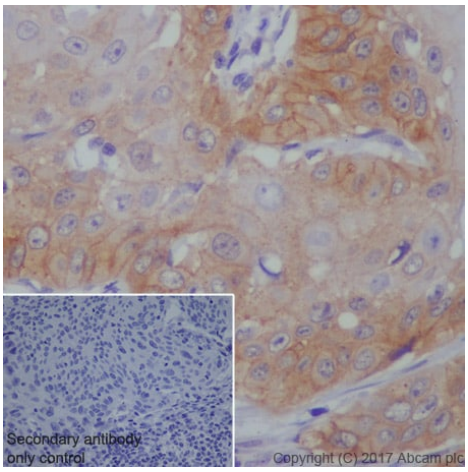
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Indirect ELISA		1/2500.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 175 kDa (predicted molecular weight: 134 kDa). Can be blocked with EGFR peptide (**ab204282**). 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
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

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).

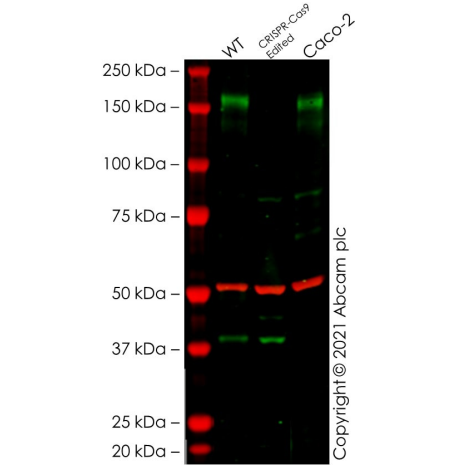
图片



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [EP38Y] - Low endotoxin, Azide free (ab174481)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human cervical carcinoma tissue sections labeling EGFR with purified **ab52894** at 1:100 dilution (0.95 µg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, pH9.0. Tissue was counterstained with Hematoxylin. **ab97051** Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52894**).



Western blot - Anti-EGFR antibody [EP38Y] - Low endotoxin, Azide free (ab174481)

All lanes : Anti-EGFR antibody [EP38Y] (**ab52894**) at 1/1000 dilution

Lane 1 : Wild-type HCT 116 cell lysate
Lane 2 : EGFR CRISPR-Cas9 edited HCT 116 cell lysate
Lane 3 : Caco-2 cell lysate

Lysates/proteins at 20 µg per lane.

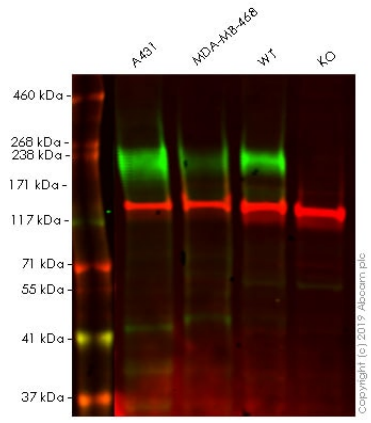
Performed under reducing conditions.

Predicted band size: 134 kDa
Observed band size: 160 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

sodium azide (**ab52894**).

False colour image of Western blot: Anti-EGFR antibody [EP38Y] 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, **ab52894** was shown to bind specifically to EGFR. A band was observed at 160 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in Egfr CRISPR-Cas9 edited cell line **ab281597** (CRISPR-Cas9 edited cell lysate **ab282949**). The band observed in the CRISPR-Cas9 edited lysate lane below 160 kDa is likely to represent a truncated form of EGFR. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and Egfr CRISPR-Cas9 edited HCT 116 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 (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-EGFR antibody [EP38Y] - Low endotoxin, Azide free (ab174481)

All lanes : Anti-EGFR antibody [EP38Y] (**ab52894**) at 1/1000 dilution

Lane 1 : A431 cell lysate
Lane 2 : MDA-MB-468 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.

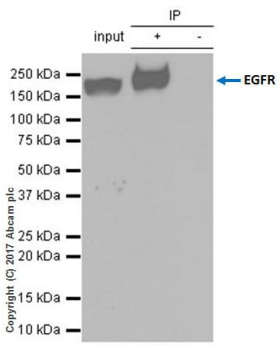
Predicted band size: 134 kDa
Observed band size: 134 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab52894**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab52894**

observed at 175 kDa. Red - loading control, **ab130007** observed at 125 kDa.

ab52894 was shown to react with EGFR in wild-type HeLa. Loss of signal was observed when knockout cell line **ab255385** (knockout cell lysate **ab263845**) was used. Wild-type and EGFR knockout samples were subjected to SDS-PAGE. **ab52894** and Anti-Vinculin antibody [VIN-54] (**ab130007**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunoprecipitation - Anti-EGFR antibody [EP38Y] - Low endotoxin, Azide free (ab174481)

ab52894 (purified) at 1:20 dilution (0.5ug) immunoprecipitating EGFR in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10ug

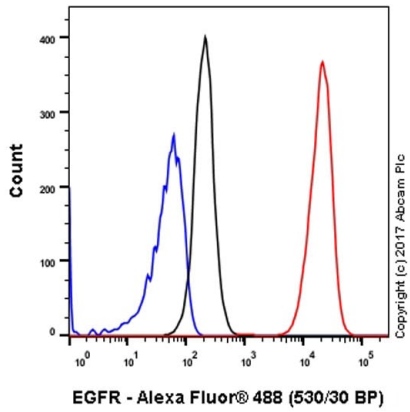
Lane 2 (+): ab52894 & HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab52894** in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.

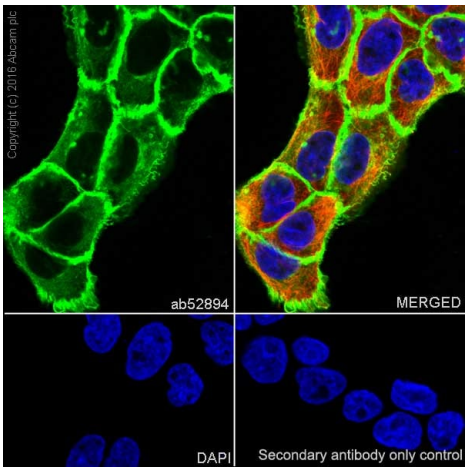
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52894**).



Flow Cytometry (Intracellular) - Anti-EGFR antibody [EP38Y] - Low endotoxin, Azide free (ab174481)

Intracellular Flow Cytometry analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labelling EGFR with purified **ab52894**. Cells were fixed with 4% Paraformaldehyde (10min) and permeabilised with 90% methanol for 30min. Then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by **ab52894** at 1/20 dilution (red) for 30 min. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).

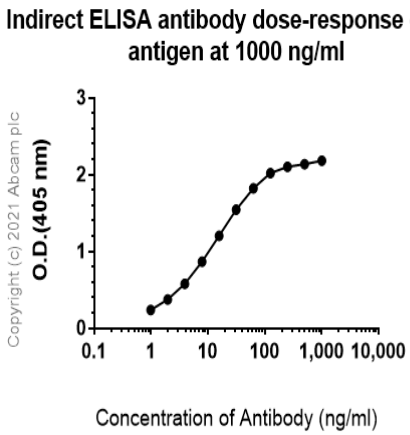
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52894**).



Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [EP38Y] - Low endotoxin, Azide free (ab174481)

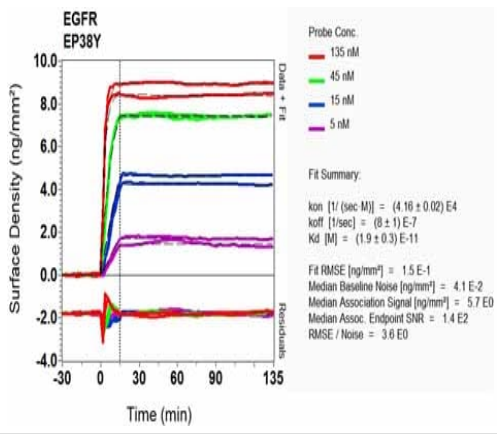
Immunocytochemistry/ Immunofluorescence analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling EGFR with Purified **ab52894** at 1:250 dilution (0.4µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52894**).



Indirect ELISA - Anti-EGFR antibody [EP38Y] - Low endotoxin, Azide free (ab174481)

ELISA analysis of Human EGFR recombinant protein at 1000 ng/ml with **ab52894**. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution was used as the secondary antibody.



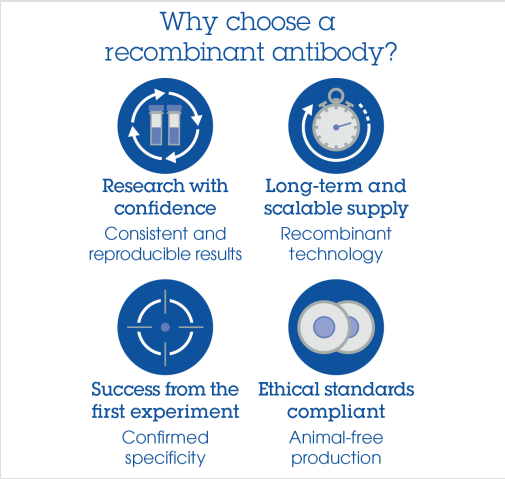
OI-RD Scanning - Anti-EGFR antibody [EP38Y] - Low endotoxin, Azide free (ab174481)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52894**).



Anti-EGFR antibody [EP38Y] - Low endotoxin, Azide free (ab174481)

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