

### Anti-EGFR antibody [EP38Y] ab52894

敲除验证
重组
RabMAb

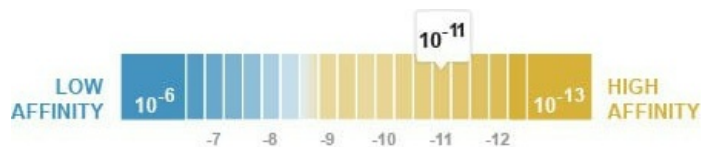
★★★★★
[24 Abreviews](#)
[300 References](#)
[16 图像](#)

#### 概述

产品名称	Anti-EGFR抗体[EP38Y]
描述	兔单克隆抗体[EP38Y] to EGFR
宿主	Rabbit
特异性	<p>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.</p> <p>The mouse and rat recommendation is based on the WB results. This antibody may not be suitable for IHC with mouse or rat samples.</p>
经测试应用	<b>适用于:</b> WB, IP, IHC-P, ICC/IF, Indirect ELISA, Flow Cyt (Intra)
种属反应性	<b>与反应:</b> Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as <a href="#">ab204282</a> )
阳性对照	ICC/IF: A431 cells. WB: HeLa, Caco-2 and A431 cell lysate; rat liver and mouse lung lysates. IP: HeLa whole cell lysate ( <a href="#">ab150035</a> ). Flow Cyt (intra): A431 cells. IHC-P: Human cervical carcinoma
常规说明	<p>Abcam recommended secondaries - Goat Anti-Rabbit HRP (<a href="#">ab205718</a>) and Goat Anti-Rabbit Alexa Fluor® 488 (<a href="#">ab150077</a>). Or search our wide range of secondary antibodies for use with your experiment.</p> <p>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb® patents</a>.</p>

#### 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. Avoid freeze / thaw cycle.
解离常数 (K <sub>D</sub> )	K <sub>D</sub> = 1.90 x 10 <sup>-11</sup> M



[Learn more about K<sub>D</sub>](#)

存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
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 ab52894 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
WB	★★★★★ (12)	1/1000 - 1/10000. Detects a band of approximately 175 kDa (predicted molecular weight: 134 kDa). Can be blocked with <b>EGFR peptide (ab204282)</b> . 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
IP		1/20.
IHC-P	★★★★★ (6)	1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. The mouse and rat recommendation is based on the WB results. <b>This antibody may not be suitable for IHC with mouse or rat samples.</b>
ICC/IF	★★★★★ (3)	1/250 - 1/500.
Indirect ELISA		1/2500.
Flow Cyt (Intra)		1/20. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

## 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
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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.

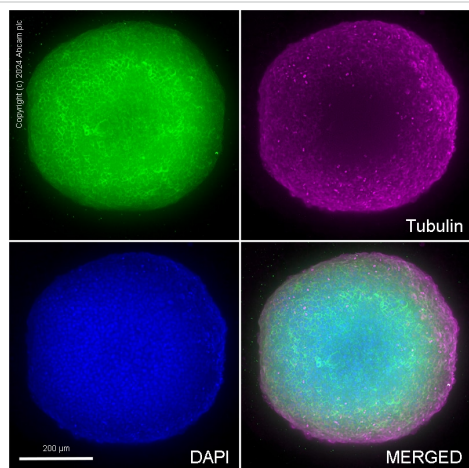
Phosphorylation at Thr-678 and Thr-693 by PRKD1 inhibits EGF-induced MAPK8/JNK1 activation. Dephosphorylation by PTPRJ prevents endocytosis and stabilizes the receptor at the plasma membrane. Autophosphorylation at Tyr-1197 is stimulated by methylation at Arg-1199 and enhances interaction with PTPN6. 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

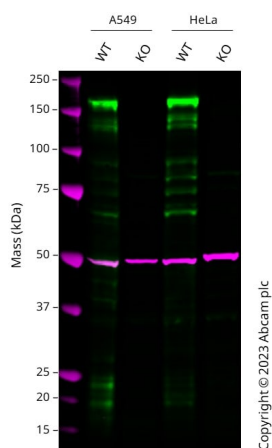


Immunocytochemistry - Anti-EGFR antibody  
[EP38Y] (ab52894)

ab52894 staining of EGFR in a HCT116 cell spheroid. The cells were fixed with 4% formaldehyde (10 min), permeabilised with 0.5% Triton X-100 for 1h and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween overnight at room temperature. The spheroids were then incubated overnight at room temperature with ab52894 at 2 μg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin at 2 μg/ml. DAPI was used as nuclear counterstain (shown in blue). As secondary antibodies **ab150081** Goat anti-Rabbit (Alexa Fluor® 488) (shown in green) and **ab150120** Goat anti-Mouse (Alexa Fluor® 594) (shown in magenta) were used, incubated overnight at room temperature. All permeabilization, blocking and antibody incubation steps were performed using a rotary shaker.

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

The antibody ab52894 also worked using 100% methanol (5 min).



Western blot - Anti-EGFR antibody [EP38Y]  
(ab52894)

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

**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.

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 134 kDa

**Observed band size:** 175 kDa

Western blot: Anti-EGFR antibody [EP38Y] (ab52894) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab52894 was shown to bind specifically to EGFR. A band was observed at 175 kDa in wild-type A549 cell lysates with no signal observed at this size in EGFR knockout cell line. To generate this image, wild-type and EGFR knockout A549 cell lysates were analysed. First, samples were run on an SDS-

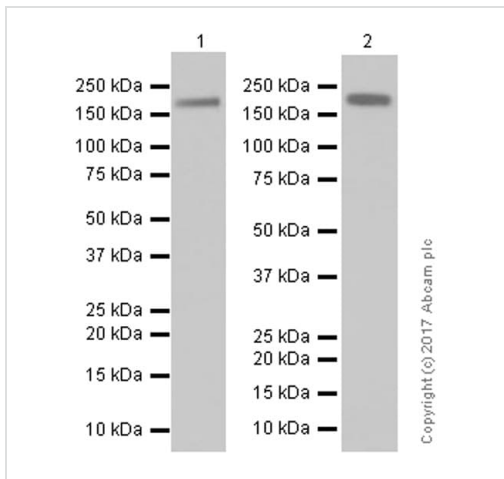
PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% milk in TBS-0.1% Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four 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.

Tissue Microarray (TMA) data for ab52894

Normal tissue samples			Malignant tissue samples		
Human cardiac muscle	×	Human placenta	✓	Clear cell carcinoma of human kidney	✓
Human cerebellum	×	Human skeletal muscle	×	Human bladder cancer	✓
Human colon	✓	Human skin	✓	Human breast carcinoma	✓
Human endometrium	✓	Human spleen	✓	Human cervical carcinoma	✓
Human kidney	✓	Human stomach	✓	Human colon carcinoma	✓
Human liver	✓	Human testis	✓	Human endometrial carcinoma	✓
Human lung	✓	Human thyroid	✓	Human gastric adenocarcinoma	✓
Human mammary gland	✓	Human tonsil	✓ (epithelial cells ✓)	Human glioma	✓
Human pancreas	✓			Human hepatocellular carcinoma	✓
				Human lung carcinoma	✓
				Human ovarian carcinoma	✓
				Human pancreatic carcinoma	✓
				Human prostatic hyperplasia	✓
				Human thyroid carcinoma	✓

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [EP38Y] (ab52894)

Tissue microarrays stained for Anti-EGFR antibody [EP38Y] using ab52894 in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. The section was incubated with ab52894 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument



Western blot - Anti-EGFR antibody [EP38Y] (ab52894)

**All lanes :** Anti-EGFR antibody [EP38Y] (ab52894) at 1/10000 dilution (purified)

**Lane 1 :** Rat liver lysates

**Lane 2 :** Mouse lung lysates

Lysates/proteins at 15 µg per lane.

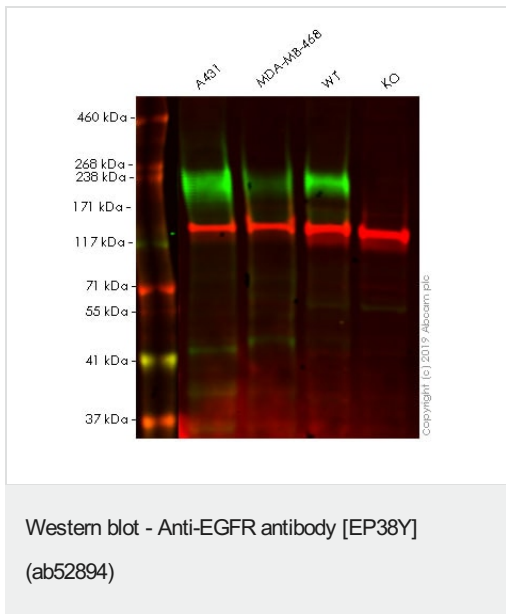
### Secondary

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

**Predicted band size:** 134 kDa

**Observed band size:** 175 kDa

Blocking and diluting buffer: 5% NFDM/TBST



**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

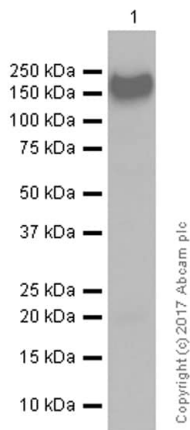
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 134 kDa

**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.



Western blot - Anti-EGFR antibody [EP38Y]  
(ab52894)

Anti-EGFR antibody [EP38Y] (ab52894) at 1/2000 dilution (purified)  
+ HeLa (Human cervix adenocarcinoma epithelial cell) whole cell  
lysates at 20 µg

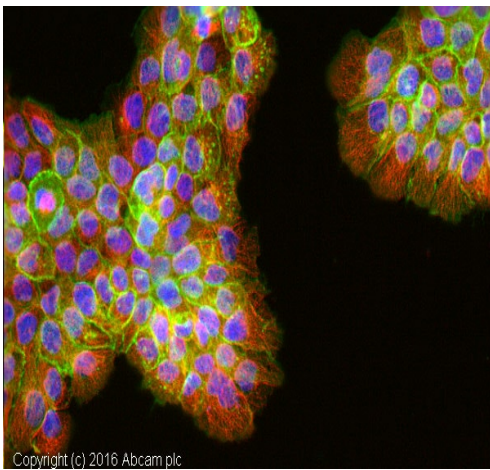
### Secondary

Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

**Predicted band size:** 134 kDa

**Observed band size:** 175 kDa

Blocking and diluting buffer: 5% NFDM/TBST



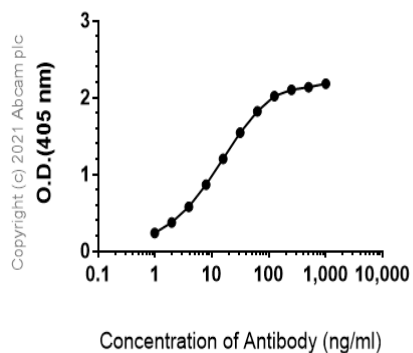
Immunocytochemistry/ Immunofluorescence - Anti-  
EGFR antibody [EP38Y] (ab52894)

Unpurified ab52894 stained A431 (Human epidermoid carcinoma cell line) cells.

The cells were fixed in 100% methanol for 5 mins at -20°C and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1 hour at room temperature to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab52894 at 1 in 500) 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 1 hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1 hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43 µM for 1 hour at room temperature.

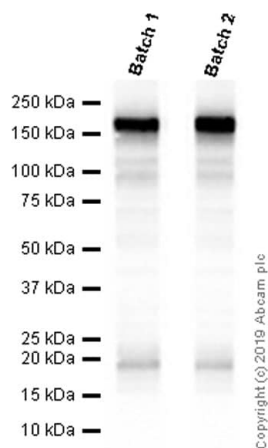


### Indirect ELISA antibody dose-response curve antigen at 1000 ng/ml



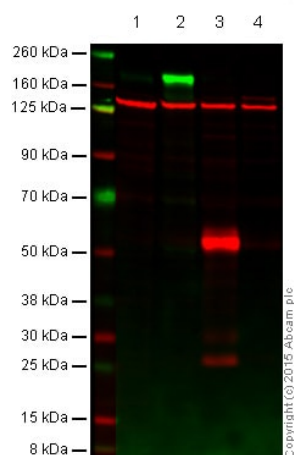
Indirect ELISA - Anti-EGFR antibody [EP38Y]  
(ab52894)

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.



Western blot - Anti-EGFR antibody [EP38Y]  
(ab52894)

Different batches of ab52894 were tested on HeLa (Human cervix adenocarcinoma epithelial cell) lysate at 1.0 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 175 kDa.



Western blot - Anti-EGFR antibody [EP38Y]  
(ab52894)

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

**Lane 1 :** Caco-2 (Human colorectal adenocarcinoma cell line) cell lysate

**Lane 2 :** A431 (Human epidermoid carcinoma cell line) cell lysate

**Lane 3 :** Mouse skin cell lysate

**Lane 4 :** Rat skin cell lysate

Lysates/proteins at 20 µg per lane.

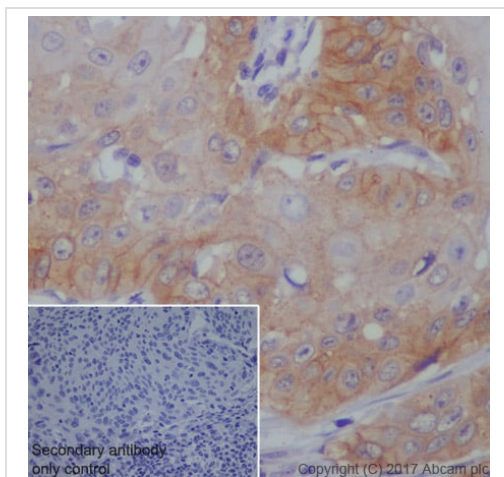
Performed under reducing conditions.

**Predicted band size:** 134 kDa



This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 mins before being transferred onto a Nitrocellulose membrane at 30V for 70 mins. The membrane was then blocked for an hour before being incubated with unpurified ab52894 overnight at 4°C in the presence of loading control **ab18058** (Mouse monoclonal [SPM227] to Vinculin diluted 1:10000). Antibody binding was detected using IR-labeled goat anti-Rabbit Ab at a 1:10,000 dilution for one hour at room temperature before imaging.

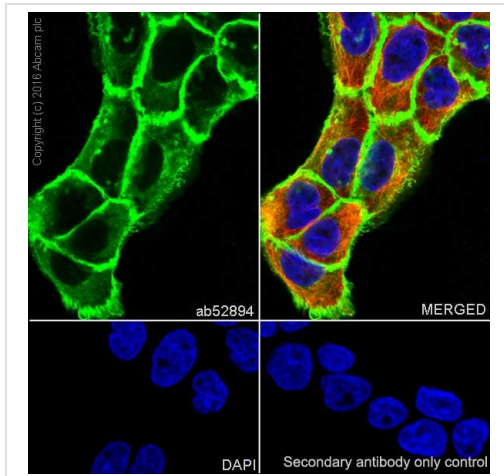
This product yielded a strong signal in western blot using A431 (human squamous carcinoma) lysate which naturally overexpresses the EGFR protein. Western blot conditions may need to be optimised for cell lines and tissues that express lower levels of endogenous EGFR.



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, pH 9.0. 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.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [EP38Y] (ab52894)

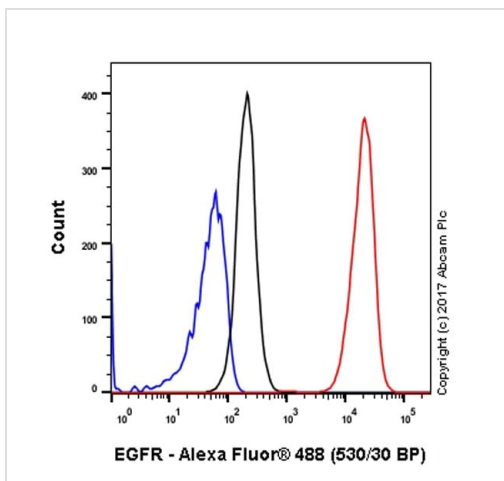


Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [EP38Y] (ab52894)

Immunocytochemistry/ Immunofluorescence analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling EGFR with purified ab52894 at 1:250 dilution (0.4 µg/ml).

Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-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.

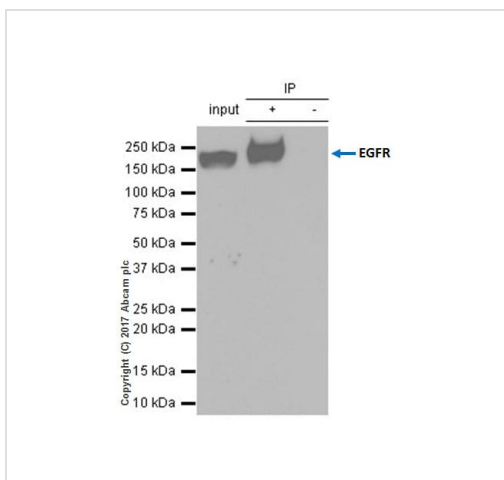
PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-EGFR antibody [EP38Y] (ab52894)

Intracellular Flow Cytometry analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling EGFR with purified ab52894.

Cells were fixed with 4% paraformaldehyde (10 mins) and permeabilized with 90% methanol for 30 mins. 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 mins. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunoprecipitation - Anti-EGFR antibody [EP38Y] (ab52894)

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

**Lane 1 (input):** HeLa whole cell lysate 10 µg

**Lane 2 (+):** ab52894 in HeLa whole cell lysate

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

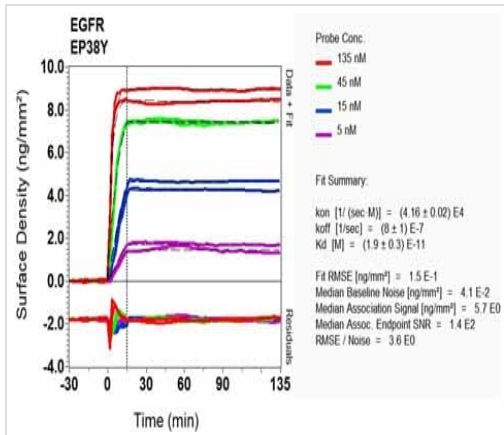
For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.

Equilibrium dissociation constant ( $K_D$ )

Learn more about  $K_D$

[Click here to learn more about  \$K\_D\$](#)



SPR Scanning - Anti-EGFR antibody [EP38Y]  
(ab52894)

Why choose a recombinant antibody?

**Research with confidence**  
Consistent and reproducible results

**Long-term and scalable supply**  
Recombinant technology

**Success from the first experiment**  
Confirmed specificity

**Ethical standards compliant**  
Animal-free production

Anti-EGFR antibody [EP38Y] (ab52894)

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