abcam

Product datasheet

Anti-EGFR antibody [EP38Y] ab52894

KO VALIDATED Recombinant RabMAb

24 Abreviews 300 References 16 Images

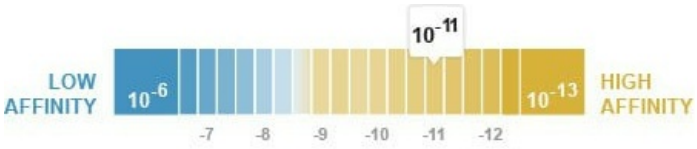
Overview

Product name	Anti-EGFR antibody [EP38Y]
Description	Rabbit monoclonal [EP38Y] to EGFR
Host species	Rabbit
Specificity	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.
Tested applications	**Suitable for:** WB, IP, IHC-P, ICC/IF, Indirect ELISA, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab204282**)
Positive control	ICC/IF: A431 cells. WB: HeLa, Caco-2 and A431 cell lysate; rat liver and mouse lung lysates. IP: HeLa whole cell lysate (**ab150035**). Flow Cyt (intra): A431 cells. IHC-P: Human cervical carcinoma
General notes	Abcam recommended secondaries - Goat Anti-Rabbit HRP (**ab205718**) and Goat Anti-Rabbit Alexa Fluor® 488 (**ab150077**). See other **anti-rabbit secondary antibodies** that can be used with this antibody. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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. Avoid freeze / thaw cycle.

Dissociation constant (K_D) $K_D = 1.90 \times 10^{-11}$ M



Learn more about K_D

Storage buffer
pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EP38Y

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab52894 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

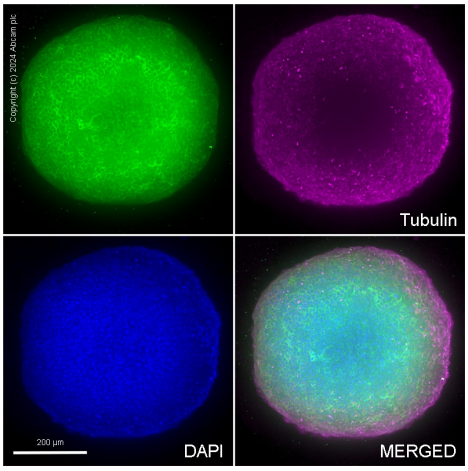
Application	Abreviews	Notes
WB	★★★★★ (12)	1/1000 - 1/10000. Detects a band of approximately 175 kDa (predicted molecular weight: 134 kDa).Can be blocked with **EGFR peptide (ab204282)**. This product yielded a strong signal in western blot using A431 (human squamous carcinoma) lysate which naturally overexpresses the EGFR protein. Western blot conditions may need to be optimised for cell lines and tissues that express lower
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. **This antibody may not be suitable for IHC with mouse or rat samples.**
ICC/IF	★★★★☆ (3)	1/250 - 1/500.
Indirect ELISA		1/2500.
Flow Cyt (Intra)		1/20. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

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

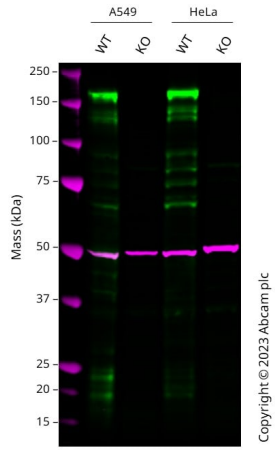


Immunocytochemistry - Anti-EGFR antibody [EP38Y] (ab52894)

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.

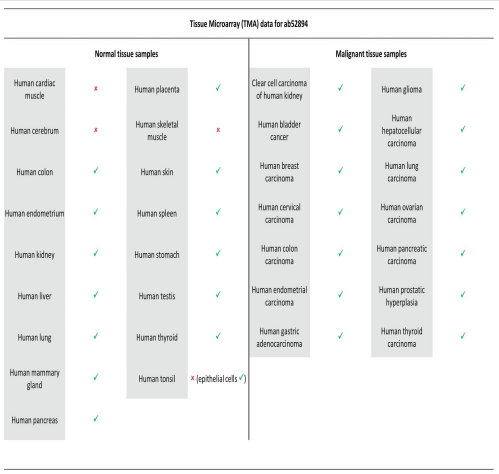
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 134 kDa
Observed band size: 175 kDa

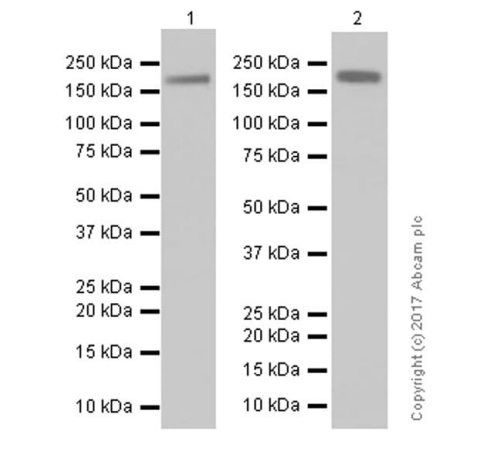
Western blot: Anti-EGFR antibody [EP38Y] (ab52894) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab52894 was shown to bind specifically to EGFR. A band was observed at 175 kDa 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.



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)

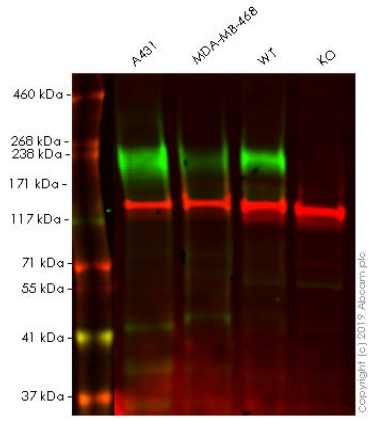
Lane 1 : Rat liver lysates
Lane 2 : Mouse lung lysates

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



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

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

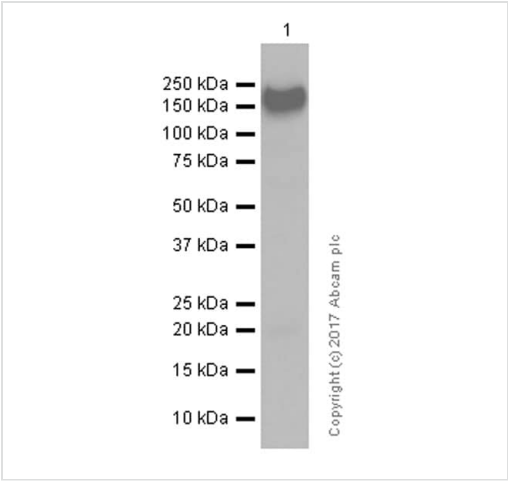
Lysates/proteins at 20 µg per lane.

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)

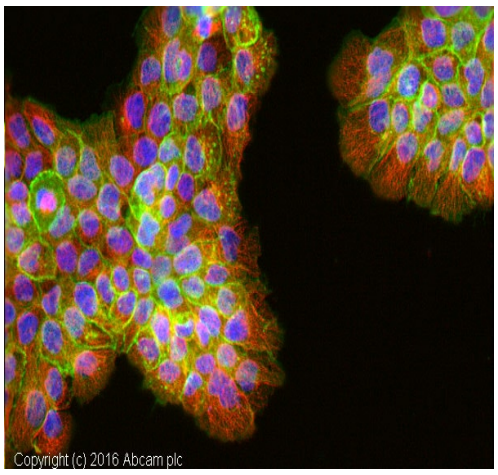
Anti-EGFR antibody [EP38Y] (ab52894) at 1/2000 dilution (purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 20 µg

Secondary

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

Predicted band size: 134 kDa
Observed band size: 175 kDa

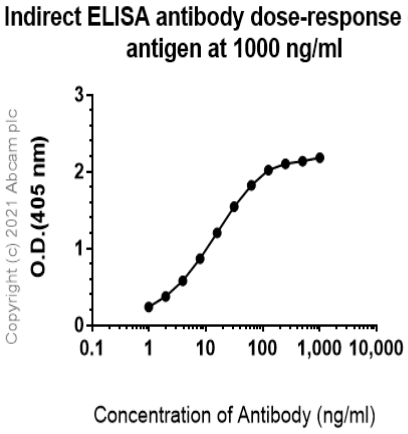
Blocking and diluting buffer: 5% NFDM/TBST



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

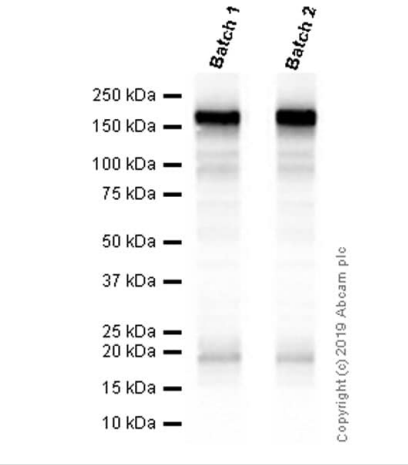
Unpurified ab52894 stained A431 (Human epidermoid carcinoma cell line) cells.

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.



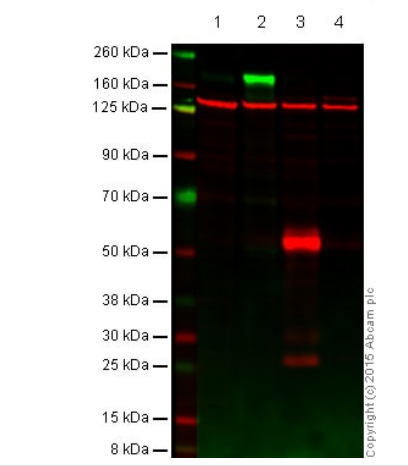
Indirect ELISA - Anti-EGFR antibody [EP38Y] (ab52894)

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

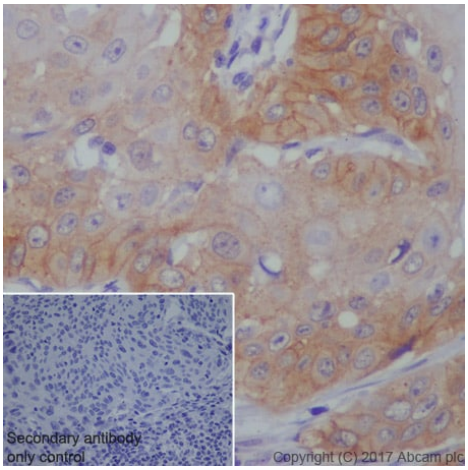
Lysates/proteins at 20 µg per lane.

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.

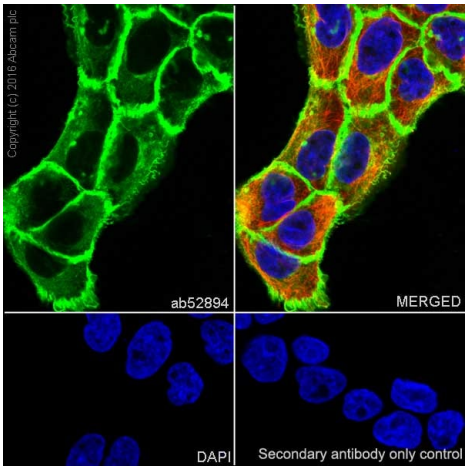
This product yielded a strong signal in western blot using A431 (human squamous carcinoma) lysate which naturally overexpresses the EGFR protein. Western blot conditions may need to be optimised for cell lines and tissues that express lower levels of endogenous EGFR.



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

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.

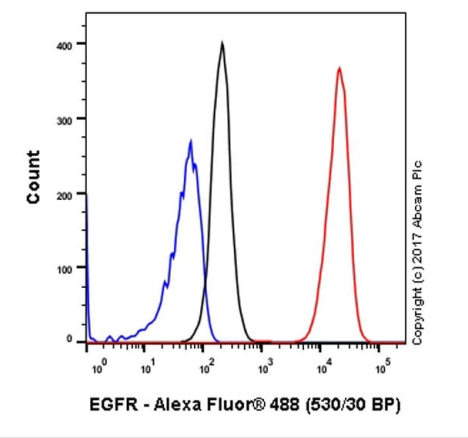


Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [EP38Y] (ab52894)

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.

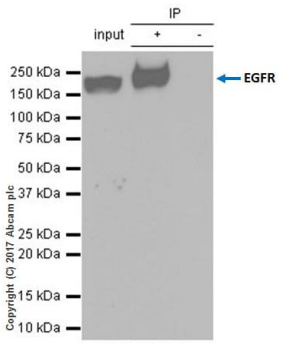
PBS instead of the primary antibody was used as the secondary antibody only control.



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 Fluorr® 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.

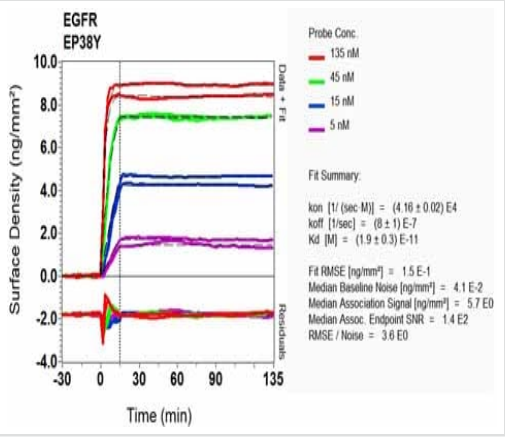
Lane 1 (input): HeLa whole cell lysate 10 µg

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.



OI-RD Scanning - Anti-EGFR antibody [EP38Y] (ab52894)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

Anti-EGFR antibody [EP38Y] (ab52894)

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