### Anti-EGFR antibody [EP38Y] ab52894

**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, Flow Cyt, IHC-P, ICC/IF, IHC-Fr |

**Species reactivity**

**Reacts with**: Mouse, Rat, Human

**Immunogen**: Synthetic peptide within Human EGFR. The exact sequence is proprietary. Synthetic phospho-peptide corresponding to residues surrounding Tyr1068 of mature human EGFR.

**Database link**: P00533

(Peptide available as ab204282)

**Positive control**

**ICC/IF**: A431 cells. **WB**: HeLa, Caco-2 and A431 cell lysate; **Rat liver and skin lysate**; **Mouse lung and skin lysate** IP: HeLa whole cell lysate (ab150035). Flow Cyt: A431 cells. **IHC-P**: Human cervical carcinoma

**General notes**

A trial size is available to purchase for this antibody.

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.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.
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 x 10^{-11} M

Storage buffer
pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: PBS, 59% Glycerol, 0.05% BSA

Purity
Protein A purified

Clonality
Monoclonal

Clone number
EP38Y

Isotype
IgG

Applications

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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>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 levels of endogenous EGFR.</td>
</tr>
<tr>
<td>IP</td>
<td>⭐</td>
<td>1/20.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>⭐</td>
<td>1/20. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>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.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/250 - 1/500.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>

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

Images
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.
Paraformaldehyde-fixed, 0.05% Triton-X permeabilized mouse adult oral epithelia (frozen sections) tissue labeling EGFR (red) using ab52894 at 1/1000 dilution in immunohistochemical analysis.

A blocking step was performed using 5% Gel Block (5% normal donkey serum, 3% BSA, 8% gelatin and 0.1% Triton X-100 in 1X PBS) at 20°C. Primary antibody was incubated for 24 hours at 4°C. Secondary antibody was polyclonal Donkey anti-rabbit Rhodamine Red-X antibody at 1/500 dilution.

Tissues were micro-dissected into ice-cold 1x PBS and fixed for 30 minutes at room temperature (RT) in 4% paraformaldehyde. After washing with PBS 3 times for 10 minutes at RT, samples were equilibrated overnight at 4°C in 15% sucrose solution and then mounted in Tissue-Tek optimal cutting temperature (OCT) compound (Electron Microscopy Services). 12 μm sagittal and coronal sections were cut on a Leica CM1950 cryostat onto Fisher SuperFrost Plus slides and stored at -80°C. Samples were dried at 37°C for 30 minutes before a 1 hour incubation with gelatin block (5% normal donkey serum, 3% BSA, 8% gelatin, and 0.1 Triton X-100 in 1X PBS). Slides were incubated with primary antibodies (Rt-Ki67 and Rb-EGFR) diluted in gelatin block overnight at 4°C and washed 3 times for 5 minutes in 1X PBS at RT. Secondary antibodies (Donkey anti-rat Alexa Fluor® 488 and Donkey anti-rabbit Rhodamine Red-X) were also diluted in gelatin block and added to the slide for 2 hours at RT. DAPI (1/2000) was added to the slide for 5 minutes at RT. Samples were mounted in 100 μl ProLong Gold and covered by glass coverslips.

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.
Lane 1: A431 cell lysate (20 µg)
Lane 2: MDA-MB-468 cell lysate (20 µg)
Lane 3: HeLa wildtype cell lysate (20 µg)
Lane 4: EGFR HeLa knockout cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab52894 observed at 134 kDa. Red - loading control, ab130007 observed at 125 kDa.

ab52894 was shown to react with EGFR in HeLa wildtype. Loss of signal was observed when knockout sample 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.

Immunohistochemical anlysis of rat skin tissue (frozen sections) labeling EGFR using ab52894 at 1/500 dilution.

Tissue was fixed using acetone. Primary antibody was incubated for 16 hours at 8°C using antibody diluent ab64211. Secondary antibody was a Goat anti-Rabbit IgG H&L (Alexa Fluor®488) (ab150077).
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 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 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).

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.
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

*why is the actual band size different from the predicted?*

Blocking and diluting buffer: 5% NFDM/TBST

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

*why is the actual band size different from the predicted?*

Blocking and diluting buffer: 5% NFDM/TBST
Other - Anti-EGFR antibody [EP38Y] (ab52894)

Equilibrium disassociation constant ($K_D$)

Learn more about $K_D$

Click here to learn more about $K_D$

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.com/abpromise or contact our technical team.

Terms and conditions

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