

Product datasheet

Anti-EGFR antibody [EP38Y] ab52894

RabMAb

★★★★☆ 6 Abreviews 17 References 12 Images

Overview

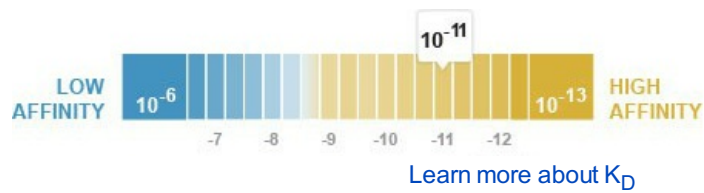
<b>Product name</b>	Anti-EGFR antibody [EP38Y]
<b>Description</b>	Rabbit monoclonal [EP38Y] to EGFR
<b>Specificity</b>	The immunogen for this product is a synthetic phospho-peptide corresponding to residues surrounding Tyr1068 of human EGFR. After screening, clone "EP38Y" was found to recognize total 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.
<b>Tested applications</b>	<b>Suitable for:</b> WB, IP, Flow Cyt, IHC-P, ICC/IF
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) corresponding to Human EGFR. Synthetic phospho-peptide corresponding to residues surrounding Tyr1068 of mature human EGFR. Database link: <a href="#">P00533</a>
<b>Positive control</b>	A431, HeLa cell lysate or human lymph node metastasis tissue.
<b>General notes</b>	A trial size is available to purchase for this antibody.  Alternative versions available: <a href="#">Anti-EGFR antibody (Alexa Fluor® 488) [EP38Y] (ab193244)</a> <a href="#">Anti-EGFR antibody (Alexa Fluor® 647) [EP38Y] (ab192982)</a> <a href="#">Anti-EGFR antibody (HRP) [EP38Y] (ab193602)</a> <a href="#">Anti-EGFR antibody (Alexa Fluor® 594) [EP38Y] (ab207870)</a> <a href="#">Anti-EGFR antibody (Phycoerythrin) [EP38Y] (ab208753)</a>  Produced using Abcam's RabMAb® technology. RabMAb® technology is covered by the following U.S. Patents, No. 5, 675, 063 and/or 7, 429, 487.

Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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



Storage buffer	PBS 49%,Sodium azide 0.01%,Glycerol 50%,BSA 0.05%
Purity	Tissue culture supernatant
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.

Application	Abreviews	Notes
WB	★★★★★	1/1000 - 1/10000. Detects a band of approximately 175 kDa (predicted molecular weight: 134 kDa).Can be blocked with <a href="#">EGFR peptide (ab204282)</a> . 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
IP		1/50.
Flow Cyt		1/100. <a href="#">ab172730</a> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. 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	★★★★★	1/250 - 1/500.

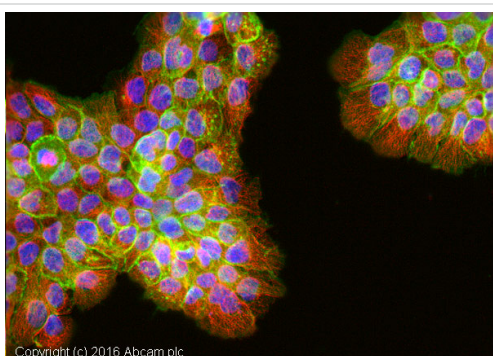
## Target

### 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.

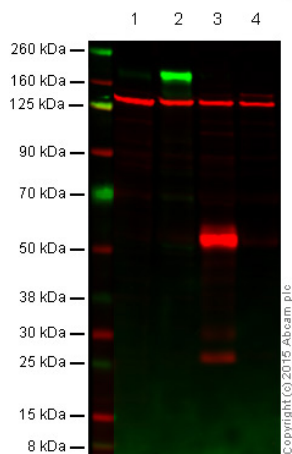
<b>Tissue specificity</b>	Isoform 2 may act as an antagonist of EGF action. Ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.
<b>Involvement in disease</b>	Lung cancer Inflammatory skin and bowel disease, neonatal, 2
<b>Sequence similarities</b>	Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 1 protein kinase domain.
<b>Post-translational modifications</b>	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.
<b>Cellular localization</b>	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).

### Anti-EGFR antibody [EP38Y] images



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

ab52894 stained A431 cells. The cells were 100% methanol fixed for 5 minutes 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 permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab52894 at 1in500) 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 1.43µM for 1 hour at room temperature.



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

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

**Lane 1** : Caco-2 cell lysate

**Lane 2** : A431 cell lysate

**Lane 3** : Mouse skin cell lysate

**Lane 4** : Rat skin cell lysate

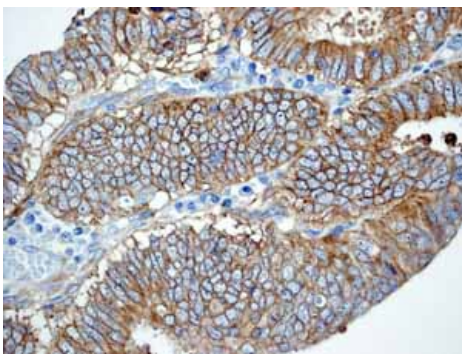
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size** : 134 kDa

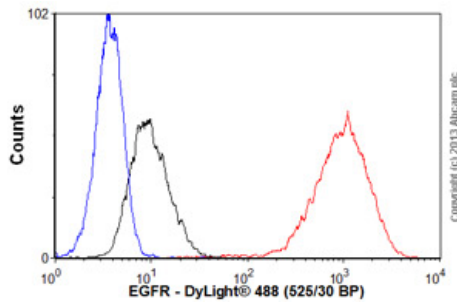
This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour before being incubated with ab52894 overnight at 4°C in the presence of loading control [ab18058](#) (Mouse monoclonal [SPM227] to Vinculin diluted 1:10000). Antibody binding was detected using IR-labelled 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.



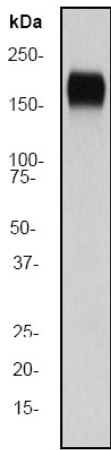
ab52894 showing positive staining in Endometrial carcinoma tissue.

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



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

Overlay histogram showing A431 cells stained with ab52894 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab52894, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was a goat anti-rabbit DyLight® 488 (IgG H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10<sup>6</sup> cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



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

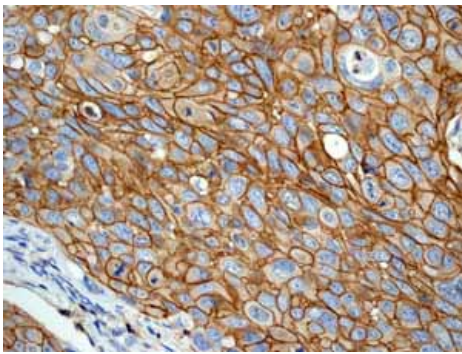
Anti-EGFR antibody [EP38Y] (ab52894) at  
1/100000 dilution + HeLa cell lysate at 10  $\mu$ g

**Secondary**

Goat anti-Rabbit HRP labeled. at 1/2000  
dilution

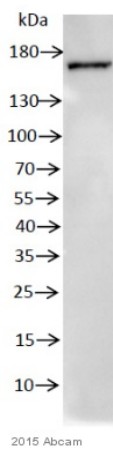
**Predicted band size** : 134 kDa

**Observed band size** : 175 kDa



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

ab52894 showing positive staining in Cervical  
carcinoma tissue.



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

Image is courtesy of an anonymous AbReview.

Anti-EGFR antibody [EP38Y] (ab52894) at 1/500 dilution + Murine macrophage lysates at 80 µg

### Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/5000 dilution

Developed using the ECL technique

Performed under reducing conditions.

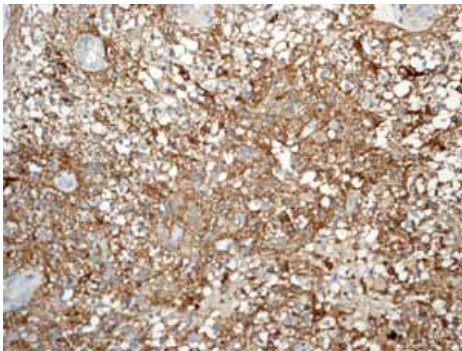
**Predicted band size :** 134 kDa

**Additional bands at :** 170 kDa (possible post-translational modification).

**Exposure time :** 1 minute

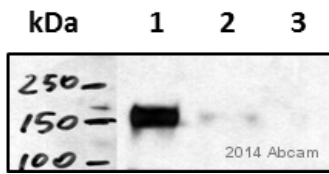
*Image is courtesy of an anonymous AbReview*

Blocking was with 5% BSA incubated for 1 hour at 25°C.



ab52894 showing positive staining in Glioma tissue.

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



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

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

**Lane 1** : H820 siCtl cell lysate, siRNA treated

**Lane 2** : H820 siEGFR#1 cell lysate, siRNA treated

**Lane 3** : H820 siEGFR#2 cell lysate, siRNA treated

Lysates/proteins at 20 µg per lane.

### Secondary

GE Healthcare Polyclonal Anti Rabbit, HRP conjugated at 1/5000 dilution

**Predicted band size** : 134 kDa

**Observed band size** : 165 kDa

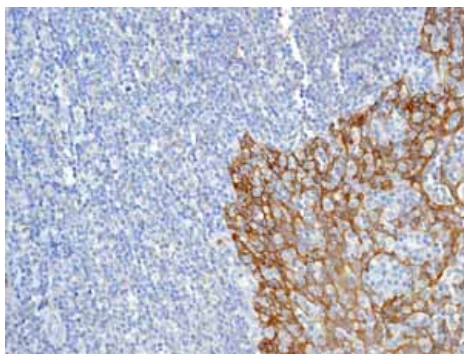
Gel running conditions: 4-15%, reduced and denatured.

Blocking agent: 5% milk.

Blocking time: 1 hour.

Incubation (with primary antibody): 16 hours, 4 Celsius.

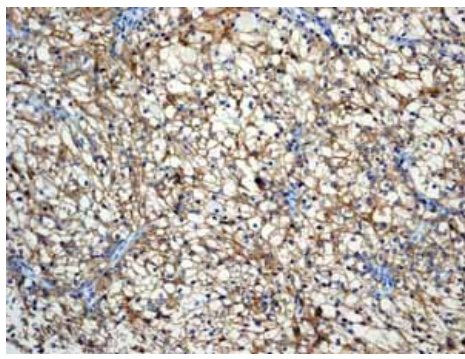
Dilution buffer: 5%BSA



ab52894 showing positive staining in Normal tonsil squamous cells tissue.

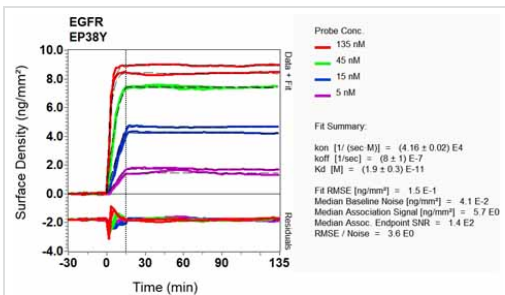
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [EP38Y] (ab52894)





ab52894 showing positive staining in Renal cell carcinoma tissue.

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



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

Equilibrium dissociation constant ( $K_D$ )

Learn more about  $K_D$

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

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