

Product datasheet

Anti-EGFR antibody [EP38Y] ab52894

RabMAb[®]

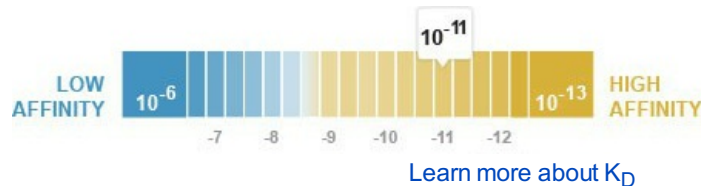
★★★★☆ 7 Abreviews 34 References 18 Images

Overview

Product name	Anti-EGFR antibody [EP38Y]
Description	Rabbit monoclonal [EP38Y] to EGFR
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
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	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: P00533
Positive control	A431, HeLa cell lysate or human lymph node metastasis tissue.
General notes	A trial size is available to purchase for 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: 40% 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.

Application	Abreviews	Notes
WB	★★★★★	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
IP		1/20. For unpurified use at 1/50.
Flow Cyt		1/20. For unpurified use at 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★★	1/100. 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- α , 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.

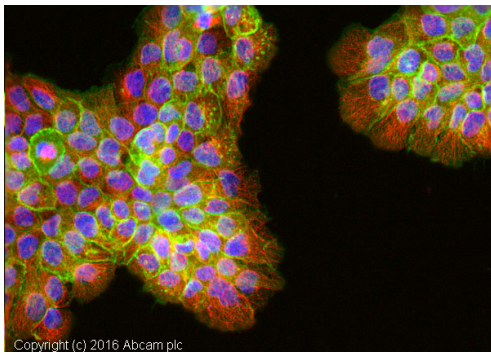
Phosphorylation at Thr-678 and Thr-693 by PRKD1 inhibits EGF-induced MAPK8/JNK1 activation. Dephosphorylation by PTPRJ prevents endocytosis and stabilizes the receptor at the plasma membrane. Autophosphorylation at Tyr-1197 is stimulated by methylation at Arg-1199 and enhances interaction with PTPN6.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

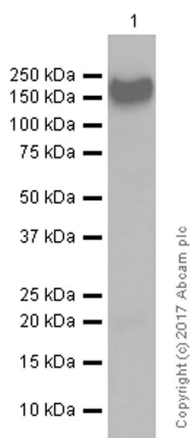
Secreted and Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus membrane. Nucleus membrane. Endosome. Endosome membrane. Nucleus. In response to EGF, translocated from the cell membrane to the nucleus via Golgi and ER. Endocytosed upon activation by ligand. Colocalized with GPER1 in the nucleus of estrogen agonist-induced cancer-associated fibroblasts (CAF).

Images



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

Unpurified 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)

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

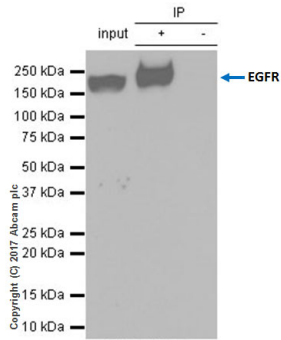
Secondary

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



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

Predicted band size : 134 kDa

Observed band size : 175 kDa

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

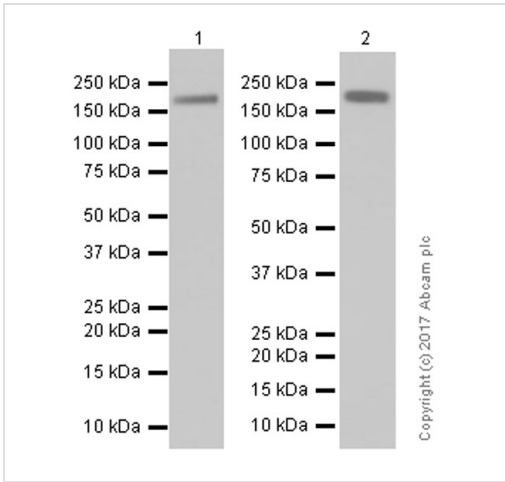
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10ug

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 secondary antibody (HRP) (ab131366) was used as the secondary antibody 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.

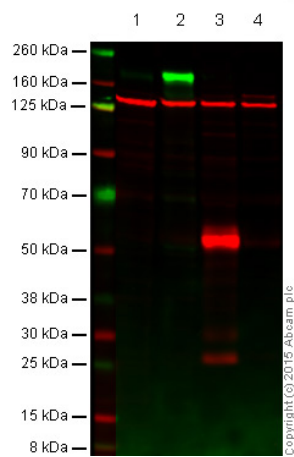
Secondary

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 (unpurified)

Lane 1 : Caco-2 cell lysate

Lane 2 : A431 cell lysate

Lane 3 : Mouse skin cell lysate

Lane 4 : Rat skin cell lysate

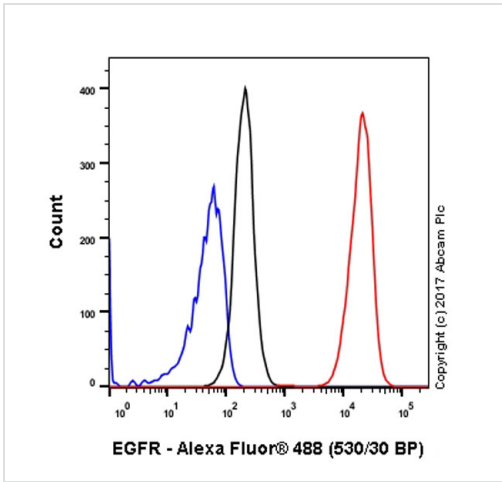
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size : 134 kDa

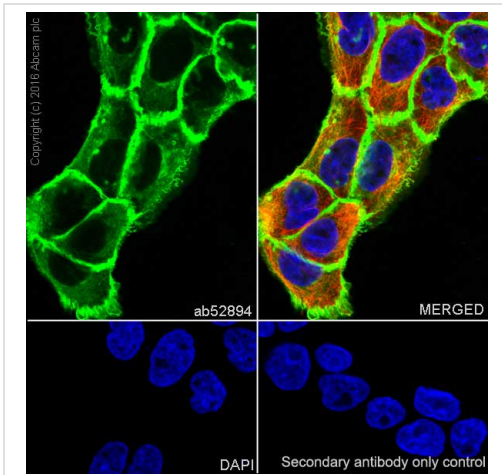
This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour before being incubated with unpurified ab52894 overnight at 4°C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



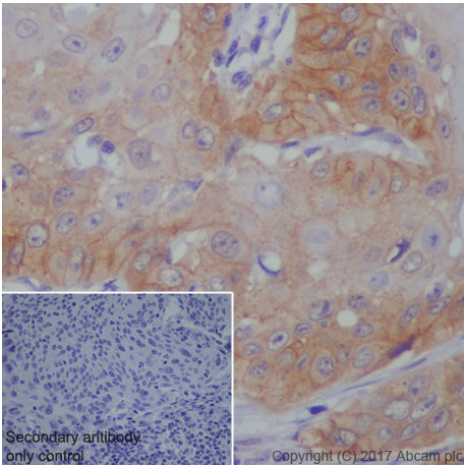
Flow Cytometry - Anti-EGFR antibody [EP38Y]
(ab52894)

Flow Cytometry analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling EGFR with purified ab52894 at 1:20 dilution (5 ug/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1:2000 dilution. Isotype control - 90% methanol. Unlabeled control - Rabbit monoclonal IgG (Black). Cell without incubation with primary antibody and secondary antibody (Blue).



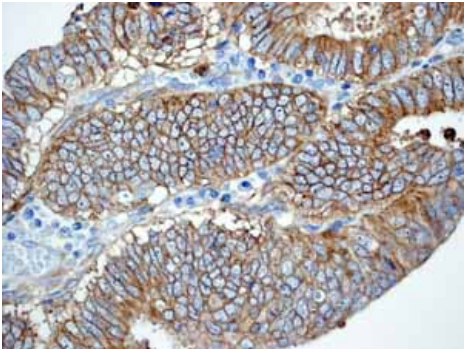
Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [EP38Y] (ab52894)

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



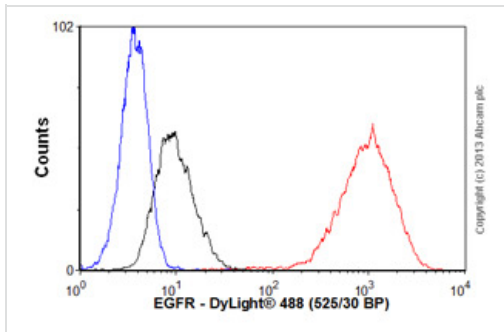
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [EP38Y] (ab52894)

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 Perform heat mediated antigen retrieval 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.



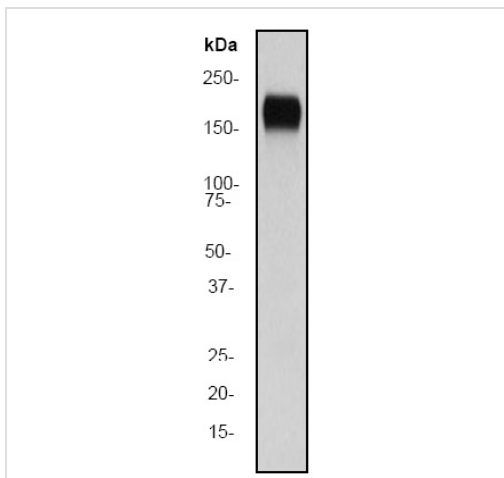
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [EP38Y] (ab52894)

Unpurified ab52894 showing positive staining in Endometrial carcinoma tissue.



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

Overlay histogram showing A431 cells stained with unpurified 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) (1 µg/1x10⁶ 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)

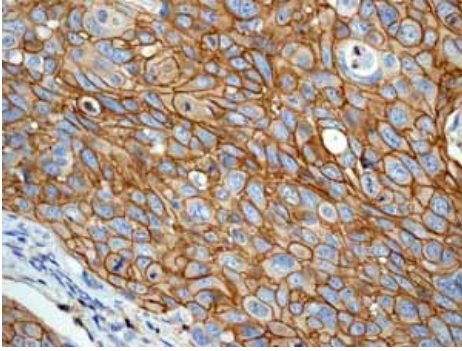
Anti-EGFR antibody [EP38Y] (ab52894) at 1/100000 dilution (unpurified) + HeLa cell lysate at 10 µg

Secondary

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

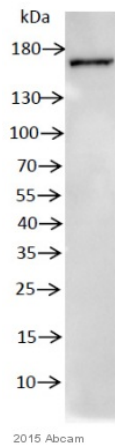
Predicted band size : 134 kDa

Observed band size : 175 kDa



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

Unpurified 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 (unpurified) + 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.

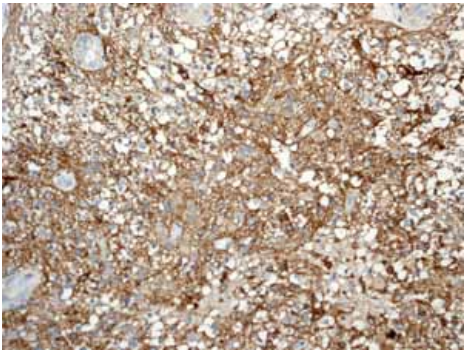
Predicted band size : 134 kDa

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

Exposure time : 1 minute

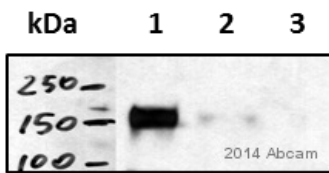
Image is courtesy of an anonymous AbReview.

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



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

Unpurified ab52894 showing positive staining in Glioma tissue.



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

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

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

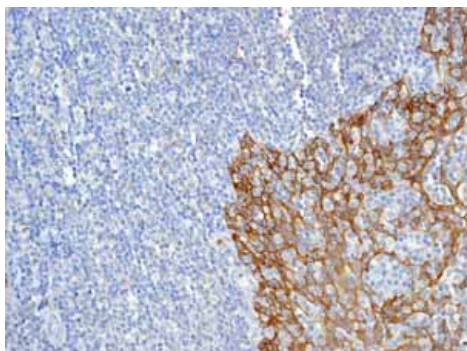
Gel running conditions: 4-15%, reduced and denatured.

Blocking agent: 5% milk.

Blocking time: 1 hour.

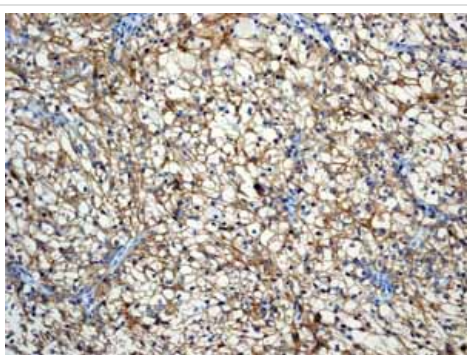
Incubation (with primary antibody): 16 hours, 4 Celsius.

Dilution buffer: 5%BSA



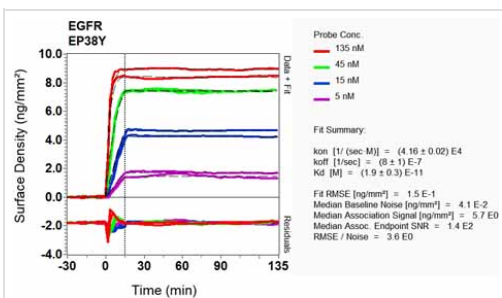
Unpurified ab52894 showing positive staining in Normal tonsil squamous cells tissue.

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



Unpurified ab52894 showing positive staining in Renal cell carcinoma tissue.

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



Equilibrium dissociation constant (K_D)

Learn more about K_D

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

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

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