abcam

Product datasheet

Anti-EGFR antibody [EP38Y] ab52894

RabMAb

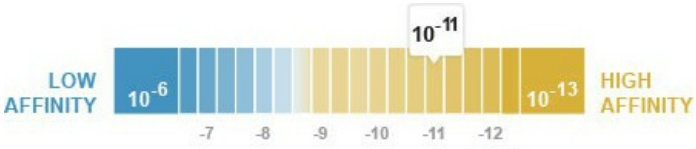
12 Abreviews 62 References 20 Images

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, Flow Cyt, IHC-P, ICC/IF, IHC-Fr
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	ICC/IF: A431 cells. WB: HeLa, Caco-2 and A431 cell lysate; Rat liver and skin lysate; Mouse lung and skin lysate; Murine macrophage lysate. IP: HeLa whole cell lysate. Flow Cyt: A431 cells. IHC-P: Human cervical carcinoma, endometrial carcinoma, glioma, tonsil and renal cell carcinoma tissue. IHC-P: Rat skin 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)	$K_D = 1.90 \times 10^{-11}$ M



Learn more about K_D

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.

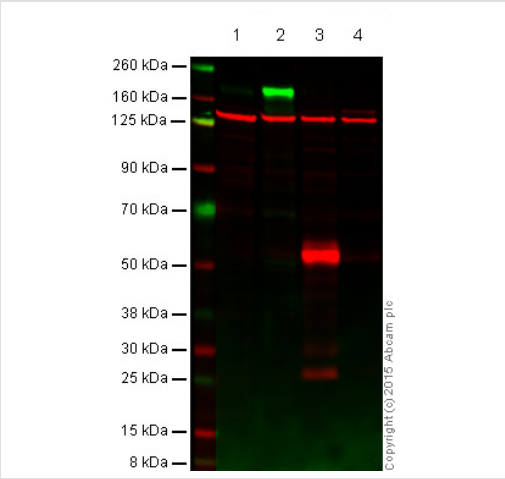
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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.
IHC-Fr	★★★★★	Use at an assay dependent concentration.

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



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

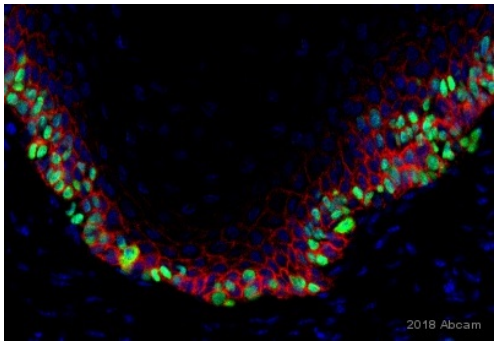
Lysates/proteins at 20 µg per lane.

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.

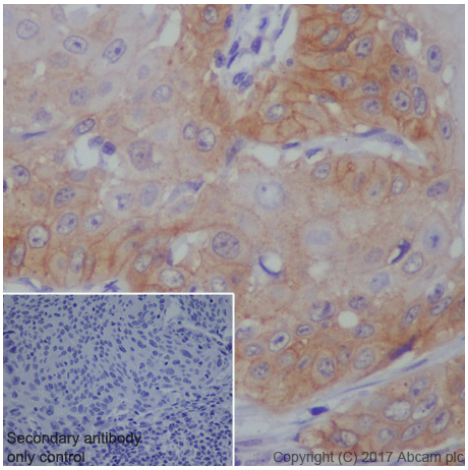


Immunohistochemistry (Frozen sections) - Anti-EGFR antibody [EP38Y] (ab52894)

Paraformadehyde-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.

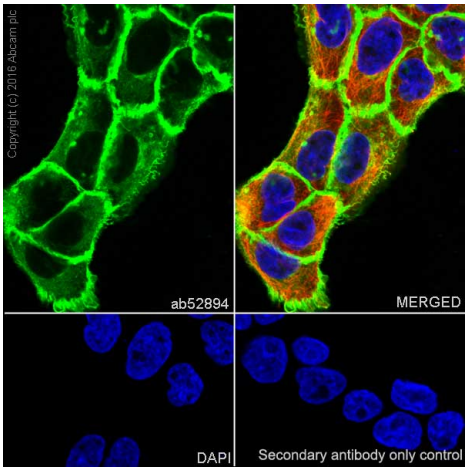
A blocking step was performed using 5% Gel Block (5% normal donkey serum, 3% BSA, 8% gelatin and 0.1% Triton X-100 in 1X PBS) at 20°C. Primary antibody was incubated for 24 hours at 4°C. Secondary antibody was polyclonal Donkey anti-rabbit Rhodamine Red-X antibody at 1/500 dilution.

Tissues were microdissected 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.



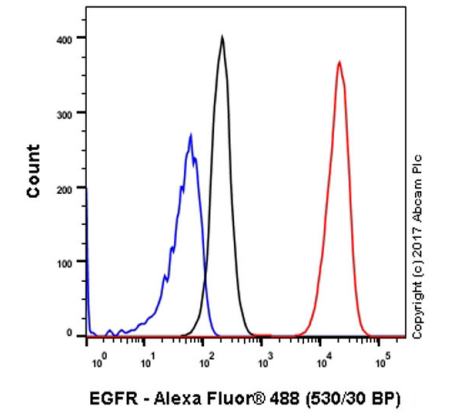
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [EP38Y] (ab52894)

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.



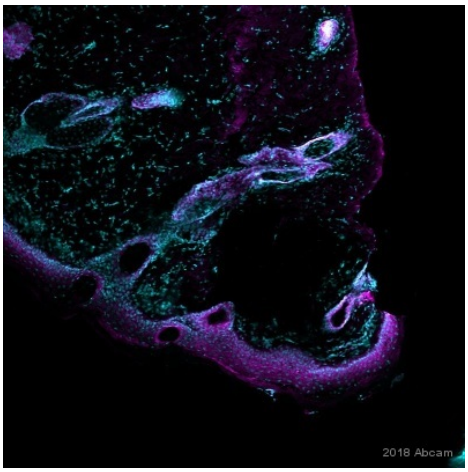
Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [EP38Y] (ab52894)

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.



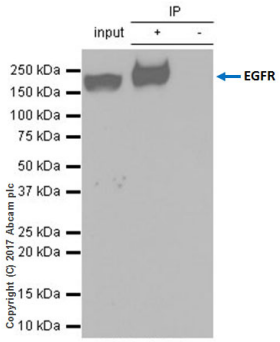
Flow Cytometry - Anti-EGFR antibody [EP38Y] (ab52894)

Flow Cytometry analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labelling EGFR with purified ab52894. Cells were fixed with 4% Paraformaldehyde (10min) and permeabilised with 90% methanol for 30min. 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 min. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1:2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Frozen sections) - Anti-EGFR antibody [EP38Y] (ab52894)

Immunohistochemical anlaysis 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).



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

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

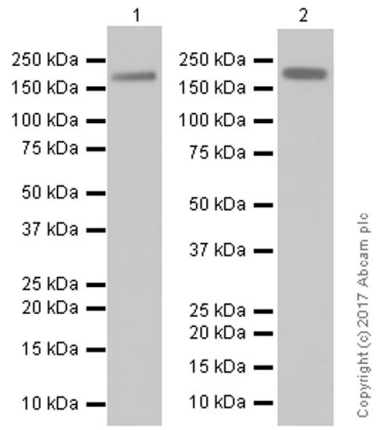
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10ug

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.

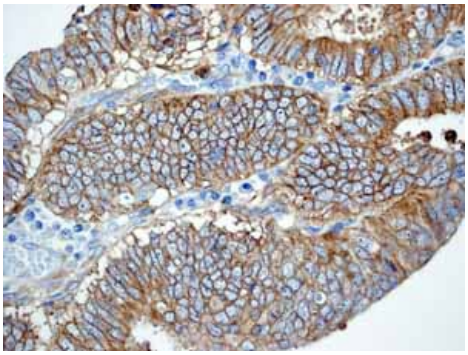
Secondary

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

Predicted band size: 134 kDa

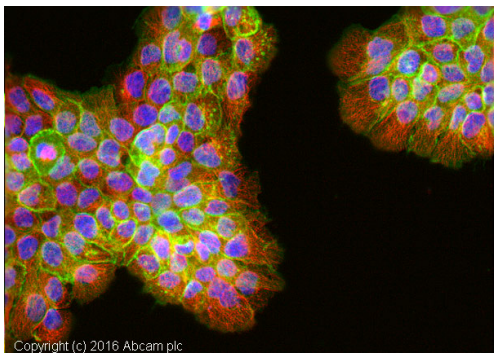
Observed band size: 175 kDa why is the actual band size different from the predicted?

Blocking and diluting buffer: 5% NFDM/TBST



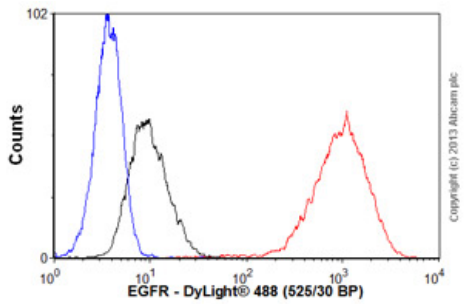
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [EP38Y] (ab52894)

Unpurified ab52894 showing positive staining in Endometrial carcinoma tissue.



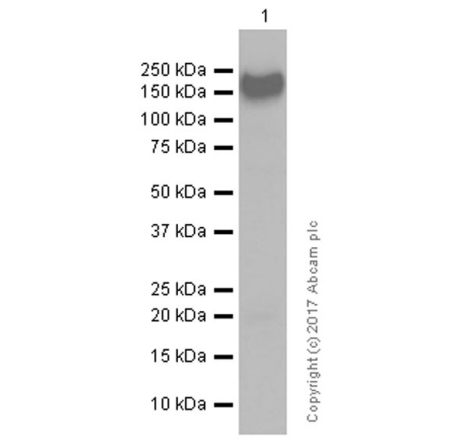
Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [EP38Y] (ab52894)

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 1hour 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 1hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.



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) (1µg/1x10^6 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 1/2000 dilution (purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 20 µg

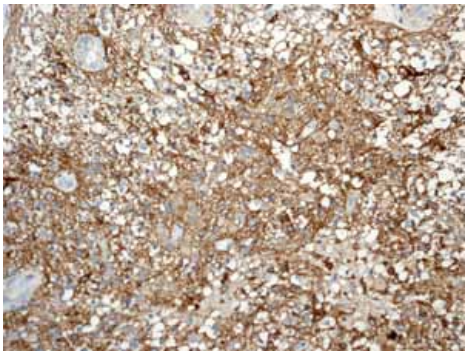
Secondary

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

Predicted band size: 134 kDa

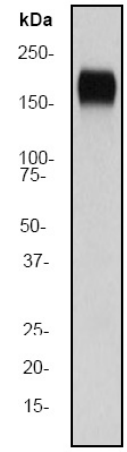
Observed band size: 175 kDa why is the actual band size different from the predicted?

Blocking and diluting buffer: 5% NFDM/TBST



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

Unpurified ab52894 showing positive staining in Glioma tissue.



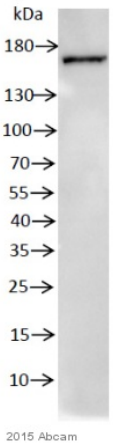
Western blot - Anti-EGFR antibody [EP38Y] (ab52894)

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 why is the actual band size different from the predicted?



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

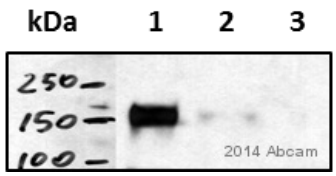
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 134 kDa
Additional bands at: 170 kDa (possible post-translational modification)

Exposure time: 1 minute

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



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

Predicted band size: 134 kDa
Observed band size: 165 kDa why is the actual band size different from the predicted?

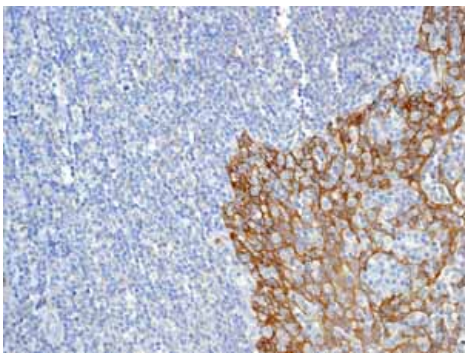
Gel running conditions: 4-15%, reduced and denatured.

Blocking agent: 5% milk.

Blocking time: 1 hour.

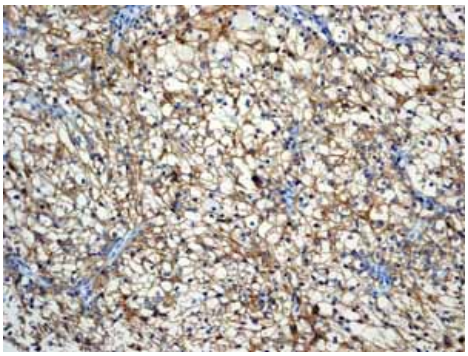
Incubation (with primary antibody): 16 hours, 4 Celsius.

Dilution buffer: 5%BSA



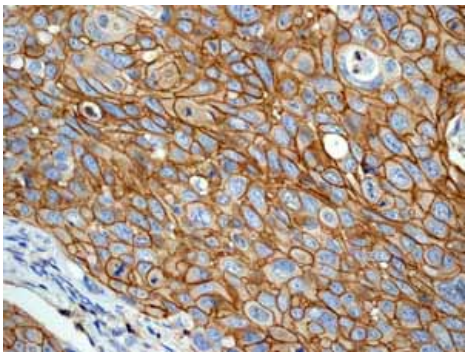
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [EP38Y] (ab52894)

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



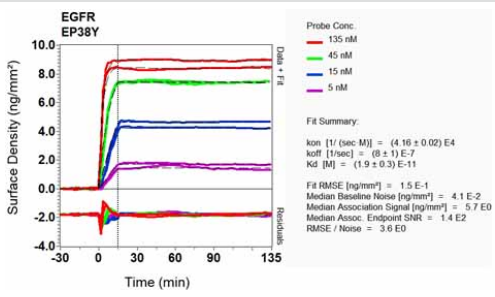
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [EP38Y] (ab52894)

Unpurified ab52894 showing positive staining in Renal cell carcinoma tissue.



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

Unpurified ab52894 showing positive staining in Cervical carcinoma tissue.



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

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

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