abcam

Product datasheet

Anti-EGFR antibody [EP38Y] - BSA and Azide free ab272293

KO VALIDATED Recombinant RabMAb

7 Images

Overview

Product name	Anti-EGFR antibody [EP38Y] - BSA and Azide free
Description	Rabbit monoclonal [EP38Y] to EGFR - BSA and Azide free
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:** Flow Cyt (Intra), ELISA, Flow Cyt, IHC-P, WB, IP, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human, Recombinant fragment **Predicted to work with:** Rat ⚠
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
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 (ab150035). 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	*Maxpar® is a trademark of Fluidigm Canada*ab272293 is the carrier-free version of ab52894. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. *Maxpar® is a trademark of Fluidigm Canada Inc.* This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP38Y
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab272293 in the following tested applications.

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

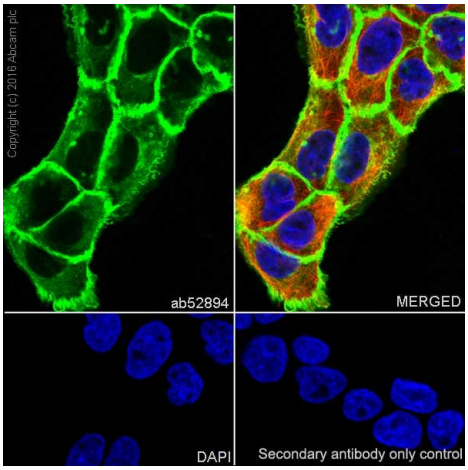
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ELISA		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 175 kDa (predicted molecular weight: 134 kDa). Can be blocked with EGFR peptide (ab204282).

Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
ICC/IF		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



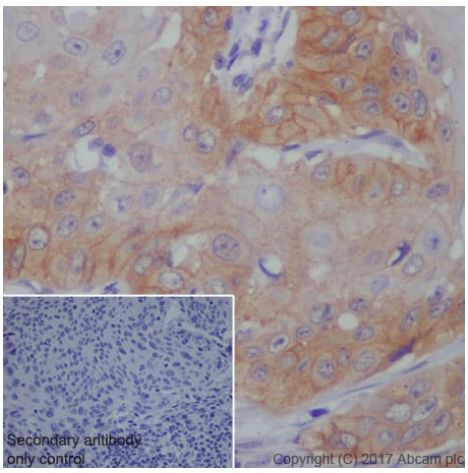
Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [EP38Y] - BSA and Azide free (ab272293)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52894).

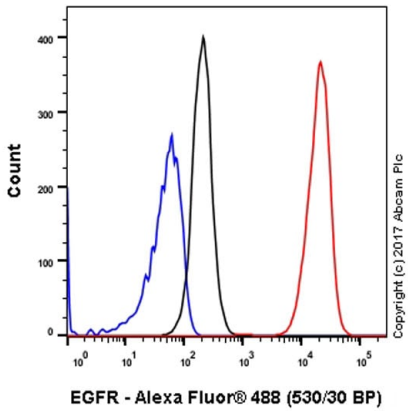


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [EP38Y] - BSA and Azide free (ab272293)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52894).

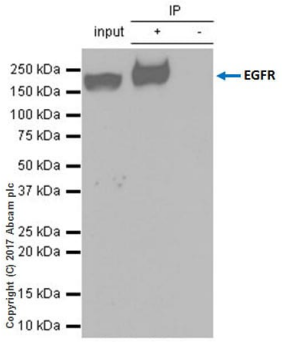


Flow Cytometry (Intracellular) - Anti-EGFR antibody [EP38Y] - BSA and Azide free (ab272293)

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52894).



Immunoprecipitation - Anti-EGFR antibody [EP38Y] - BSA and Azide free (ab272293)

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

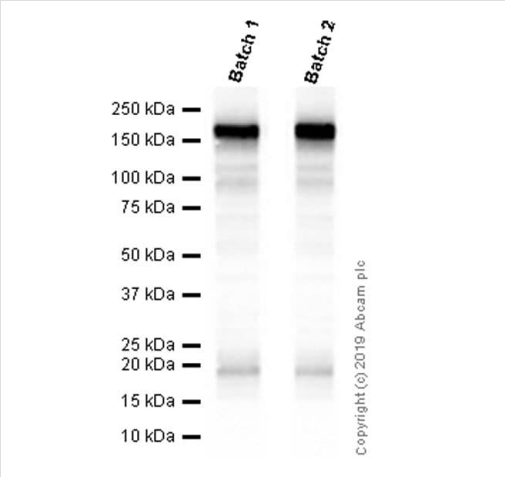
Lane 2 (+): ab52894 in HeLa whole cell lysate

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.

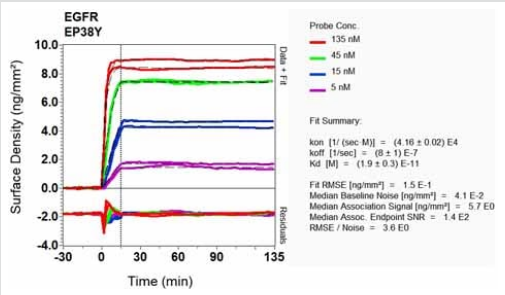
Blocking and diluting buffer: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52894).



Western blot - Anti-EGFR antibody [EP38Y] - BSA and Azide free (ab272293)

This data was developed using ab52894, the same antibody clone in a different buffer formulation. 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.



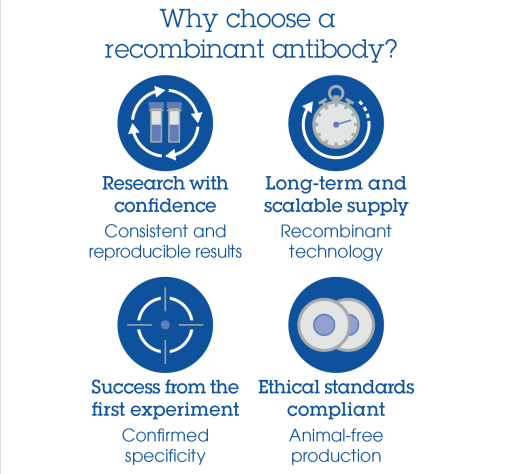
OI-RD Scanning - Anti-EGFR antibody [EP38Y] - BSA and Azide free (ab272293)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab52894).



Anti-EGFR antibody [EP38Y] - BSA and Azide free (ab272293)

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