abcam

ab182618

Anti-EGFR (phospho Y1068) antibody [EP774Y] - BSA and Azide free

Recombinant RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP774Y
Purification technique	Affinity purification Protein A
Specificity	Recognises EGFR phosphorylated on Tyrosine 1068 of the mature human isoform 1 (corresponding to Y1092 from the precursor form P00533-1/p170)
Dissociation constant	3.6 x 10^{-11} M
Concentration	1.018 - 1.053 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Dot

Tested

Species	Synthetic peptide
Dilution info	-
Notes	-

Expected

Species	Human, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	-

Notes	Can be blocked with EGFR (phospho Y1092) peptide (ab190788).

Species	Human
Dilution info	-
Notes	Can be blocked with EGFR (phospho Y1092) peptide (ab190788).

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human

Dilution info	-
Notes	-

Not recommended

Species	Mouse, Synthetic peptide
Dilution info	-
Notes	-

Target data

See full target information EGFR pY1068

Function

Receptor tyrosine kinase binding ligands of the EGF family and activating several signaling cascades to convert extracellular cues into appropriate cellular responses (PubMed:10805725, PubMed:27153536, PubMed:2790960, PubMed:35538033). Known ligands include EGF, TGFA/TGF-alpha, AREG, epigen/EPGN, BTC/betacellulin, epiregulin/EREG and HBEGF/heparin-binding EGF (PubMed:12297049, PubMed:15611079, PubMed:17909029, PubMed:20837704, PubMed:27153536, PubMed:2790960, PubMed:7679104, PubMed:8144591, PubMed:9419975). 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 (PubMed:27153536). May also activate the NF-kappa-B signaling cascade (PubMed:11116146). 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 (PubMed:11602604). Also phosphorylates MUC1 and increases its interaction with SRC and CTNNB1/beta-catenin (PubMed:11483589). Positively regulates cell migration via interaction with CCDC88A/GIV which retains EGFR at the cell membrane following ligand stimulation, promoting EGFR signaling which triggers cell migration (PubMed:20462955). Plays a role in enhancing learning and memory performance (By similarity). Plays a role in mammalian pain signaling (long-lasting hypersensitivity) (By similarity). Isoform 2 may act as an antagonist of EGF action. (Microbial infection) Acts as a receptor for hepatitis C virus (HCV) in hepatocytes and facilitates its cell entry. Mediates HCV entry by promoting the formation of the CD81-CLDN1 receptor complexes that are essential for HCV entry and by enhancing membrane fusion of cells expressing HCV envelope glycoproteins.

Storage

Shipped at conditions	Blue Ice

Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab182618 is the carrier-free version of ab40815.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

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.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	EGFR or Epidermal Growth Factor Receptor is a transmembrane glycoprotein that acts as a receptor for members of the epidermal growth factor family. Known alternatively as ErbB1 or HER1 this receptor has an approximate molecular weight of 170 kDa. EGFR is expressed in various cell types notably on epithelial cells and can influence multiple cellular processes through its kinase activity. It participates in the regulation of cell growth multiplication and survival by activating its kinase domain upon ligand binding.

Biological function summary	The EGFR protein plays an important role in cellular communication and signaling processes. EGFR pairs with other receptor family members to form active dimers or even higher-order complexes which in turn initiate intracellular signaling cascades. Through these complexes EGFR influences many processes including cell differentiation and repair. This function of EGFR makes it an integral part of mammalian biology affecting how cells respond to their environment by mediating changes in gene expression.
Pathways	EGFR is a central player in the MAPK and PI3K/Akt signaling pathways. Alongside other protein partners like KRAS and PI3 kinase it contributes to transmitting signals from the cell surface to the nucleus affecting gene transcription and cell behavior. These pathways are important for normal cell growth and division and aberrations in these pathways can lead to excessive or insufficient cell proliferation.
Associated diseases and disorders	EGFR is pertinent to cancer biology including non-small cell lung cancer and glioblastoma where mutations or overexpression of the receptor frequently occur. It connects to proteins such as PTEN and BRAF which influence tumor progression and response to targeted therapies. EGFR's involvement in these disorders highlights its significance as a therapeutic target since it can be manipulated to alter disease progression.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

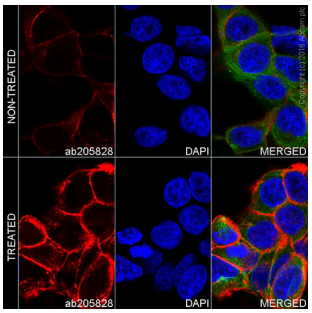
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

10 product images



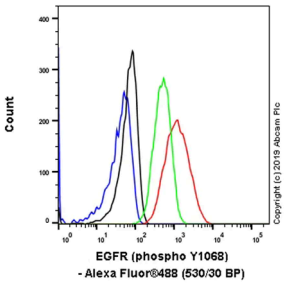
Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho Y1068) antibody [EP774Y] - BSA and Azide free (ab182618)

EGFR (phospho Y1068) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EGFR (phospho Y1068) antibody

Clone EP774Y (ab182618) has been successfully conjugated by Abcam. This image was generated using Anti-EGFR (phospho Y1068) antibody [EP774Y] (Alexa Fluor® 647). Please refer to ab205828 for protocol details.
ab205828 staining EGFR (phospho Y1092) in A431 cells +/-EGF (100ng/ml, 5min). The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h.

The cells were then incubated overnight at +4°C with ab205828 at 1:100 dilution (shown in red) and ab195887, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

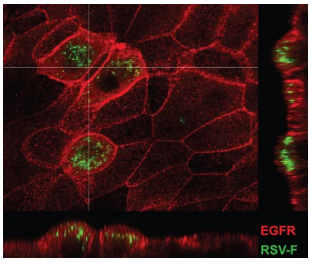
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry (Intracellular) - Anti-EGFR (phospho Y1068) antibody [EP774Y] - BSA and Azide free (ab182618)

EGFR (phospho Y1068) Flow Cytometry (Intracellular) staining using rabbit Anti-EGFR (phospho Y1068) antibody

Intracellular Flow Cytometry analysis of A431 (Human epidermoid carcinoma epithelial cell) treated with 200 ng/ml EGF for 15 minutes cells labeling EGFR with purified ab40815 at 1/800 dilution (1µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue). Untreated A431 cells (Green). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40815).



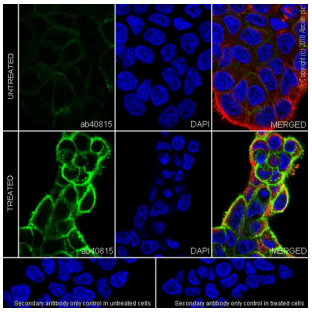
Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho Y1068) antibody [EP774Y] - BSA and Azide free (ab182618)

EGFR (phospho Y1068) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EGFR (phospho Y1068) antibody

WD-PBEC cultures were infected with RSV clinical isolate BT2a and stained for EGFR (red) and RSV F (RSV F, green) expression.
For WD-PBECs, pediatric bronchial epithelial cells (PBEC) were obtained, via written parental consent, from bronchial brushings of children undergoing elective surgery at the Royal Belfast Hospital for Sick Children, and the procedures were approved by the Office of Research Ethics Committees Northern Ireland. PBEC were expanded in collagen-coated flasks using airway epithelial cell media and supplements (Lonza), then seeded onto transwell inserts (Corning), and then air-liquid interface (ALI) cultures were initiated and maintained 21 days in order to establish well-differentiated (WD)-PBECs. Paraformaldehyde-fixed and permeabilized WD-PBEC were stained for RSV F protein expression and were stained with anti-phospho-(p)-EGFR (Abcam,

ab40815). WD-PBEC cultures were infected with RSV subgroup A clinical isolate BT2a. Fluorescent images were obtained with a SP5 confocal DMI 6000 inverted microscope (Leica).

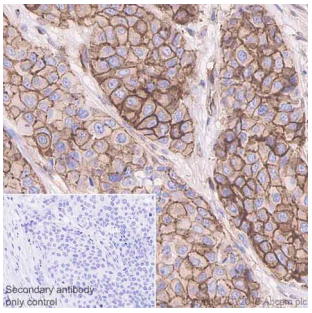
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40815).



Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho Y1068) antibody [EP774Y] - BSA and Azide free (ab182618)

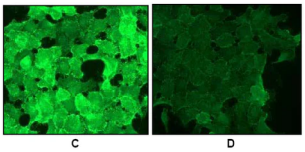
EGFR (phospho Y1068) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EGFR (phospho Y1068) antibody

Immunocytochemistry/ Immunofluorescence analysis of A431 (Human epidermoid carcinoma epithelial cell) treated with 100 ng/ml EGF for 10 minutes cells labeling EGFR with purified ab40815 at 1/500 dilution (1.8 µg/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) at 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI (blue) was used as 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 (ab40815).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR (phospho Y1068) antibody [EP774Y] - BSA and Azide free (ab182618)

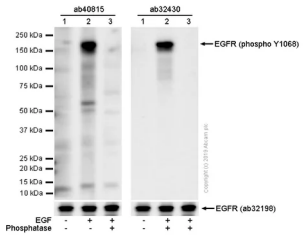
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast cancer tissue sections labeling EGFR with purified ab40815 at 1/500 dilution (1.75 µg/ml). Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40815).



Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho Y1068) antibody [EP774Y] - BSA and Azide free (ab182618)

EGFR (phospho Y1068) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EGFR (phospho Y1068) antibody

Immunofluorescent staining of untreated (C) and Phosphatase- treated (D) A431 cells using ab40815 (unpurified).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40815).



Western blot - Anti-EGFR (phospho Y1068) antibody [EP774Y] - BSA and Azide free (ab182618)

EGFR (phospho Y1068) Western blot staining using rabbit Anti-EGFR (phospho Y1068) antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40815).
This antibody detects high background.

All lanes:
Western blot - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815) at 1/1000 dilution

Lane 1:
A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates at 15 µg

Lane 2:
A431 (Human epidermoid carcinoma epithelial cell) treated with 10 ng/ml EGF for 30 minutes whole cell lysates at 15 µg

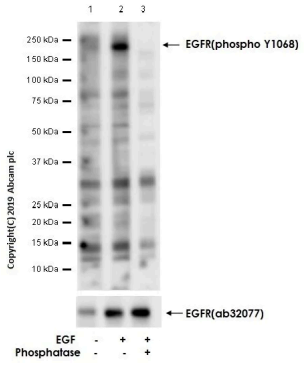
Lane 3:
A431 (Human epidermoid carcinoma epithelial cell) treated with 10 ng/ml EGF for 30 minutes. Then the membrane was incubated with phosphatase at 15 µg

Secondary

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

Predicted band size: 134 kDa

Observed band size: 175 kDa



Western blot - Anti-EGFR (phospho Y1068) antibody [EP774Y] - BSA and Azide free (ab182618)

EGFR (phospho Y1068) Western blot staining using rabbit Anti-EGFR (phospho Y1068) antibody

Blocking and dilution 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 (ab40815).
Lanes 1 - 3:
Western blot - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815) at 1/1000 dilution

Lanes 1 - 3:
Western blot - Anti-EGFR (phospho Y1068) antibody [EP774Y] - BSA and Azide free (ab182618) at 1/1000 dilution

Lane 1:
C2C12 (Mouse myoblasts myoblast) whole cell lysate at 20 µg

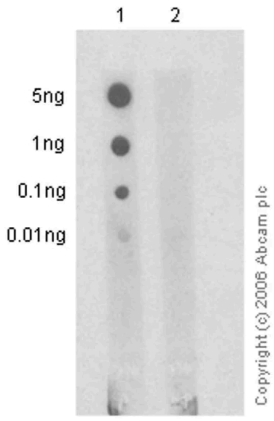
Lane 2:
C2C12 (Mouse myoblasts myoblast) treated with 100 ng/ml EGF for 24 hours whole cell lysate at 20 µg

Lane 3:
C2C12 (Mouse myoblasts myoblast) treated with 100 ng/ml EGF for 24hours whole cell lysate, then the membrane was incubated with phosphatase at 20 µg

Secondary

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

Exposure time: 30s

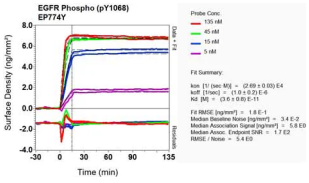


Dot Blot - Anti-EGFR (phospho Y1068) antibody [EP774Y] - BSA and Azide free (ab182618)

Dot blot analysis of EGFR (pY1068) peptide (Lane 1), SMAD5 (unmodified) peptide labelling EGFR (pY1068) with ab40815 (unpurified) at a dilution of 1/1000. Peroxidase conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody at a dilution of 1/2500.
Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40815).



OI-RD Scanning - Anti-EGFR (phospho Y1068) antibody [EP774Y] - BSA and Azide free (ab182618)

We have systematically measured KD (the equilibrium dissociation constant between the antibody and its antigen), of more than 840 recombinant antibodies to assess not only their individual KD values but also to see the average affinity of antibody. Based on the comparison with published literature values for mouse monoclonal antibodies, Recombinant antibodies appear to be on average 1-2 order of magnitude higher affinity.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.