abcam

ab227459

Anti-EGFR antibody [E235] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	E235
Purification technique	Affinity purification Protein A
Specificity	This antibody detects Epidermal growth factor receptor (EGFR). It does not cross react with other ERBB family members. 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.
Concentration	1.01 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

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

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

WB

Tested

Species	Human
Dilution info	-
Notes	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.

Species	Mouse
Dilution info	-

Notes	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.

Species	Rat
Dilution info	-
Notes	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.

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376- Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.

Notes	-

Target data

See full target information EGFR

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

ab227459 is the carrier-free version of ab32077.

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

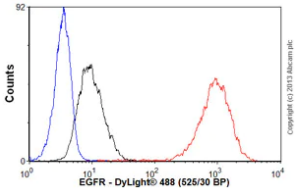
We do not recommend this combination. It is not covered by our product promise.

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.

6 product images

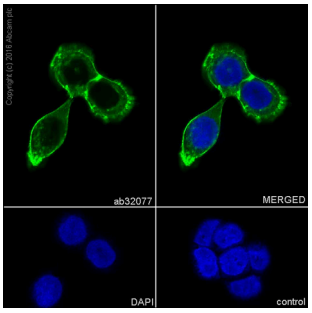


Flow Cytometry (Intracellular) - Anti-EGFR antibody [E235] - BSA and Azide free (ab227459)

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

Overlay histogram showing A431 cells stained with ab32077 (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 (ab32077, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution

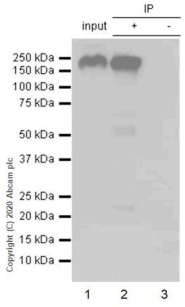
for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32077).



Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [E235] - BSA and Azide free (ab227459)

EGFR Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EGFR antibody

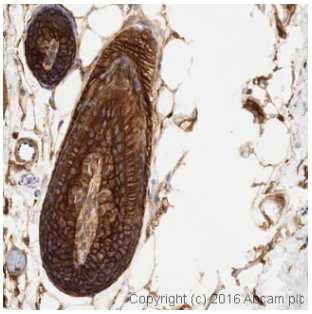
Immunocytochemistry/Immunofluorescence analysis of A431 cells labelling EGFR with ab32077 at 1/500. Cells were fixed with 100% methanol. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody.
Control: PBS only.
Nuclear counter stain: DAPI.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32077).



Immunoprecipitation - Anti-EGFR antibody [E235] - BSA and Azide free (ab227459)

Purified ab32077 at 1/50 dilution (2µg) immunoprecipitating EGFR in A431 whole cell lysate.
Lane 1 (input): A431 (Human epidermoid carcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab32077 + A431 whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32077 in A431 whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (ab131366) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 180 kDa
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32077).
All lanes:
Immunoprecipitation - Anti-EGFR antibody [E235] (ab32077)

Predicted band size: 134 kDa



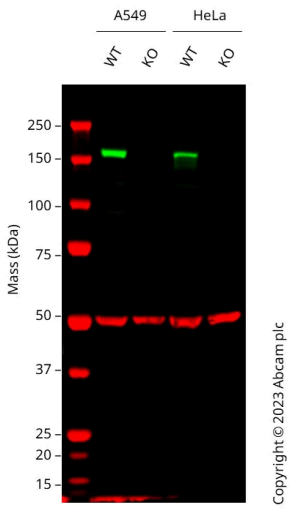
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [E235] - BSA and Azide free (ab227459)

EGFR Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-EGFR antibody

This IHC data was generated using the same anti-EGFR antibody clone, E235, in a different buffer formulation (cat# ab32077).
IHC image of EGFR staining in a formalin fixed, paraffin embedded mouse normal skin tissue section, performed on a Leica Bond™ system using the standard protocol B. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab32077 at 1/200 dilution for 15 mins

at room temperature. A goat anti-rabbit biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Western blot - Anti-EGFR antibody [E235] - BSA and Azide free (ab227459)

EGFR Western blot staining using rabbit Anti-EGFR antibody

Western blot: Anti-EGFR antibody [E235] (ab32077) staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32077 was shown to bind specifically to EGFR. A band was observed at 160 kDa in wild-type cell lysates with no signal observed at this size in EGFR knockout cell lines. To generate this image, wild-type and EGFR knockout A549 (ab286394) and HeLa (ab255385) cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% milk in TBS-0.1% Tween ® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32077).
All lanes:
Western blot - Anti-EGFR antibody [E235] (ab32077) at 1/1000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

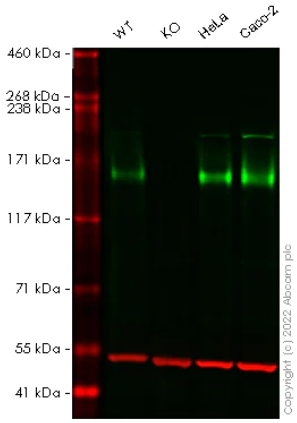
Lane 2:
EGFR knockout A549 cell lysate at 20 µg

Lane 3:
Wild-type HeLa cell lysate at 20 µg

Lane 4:
EGFR knockout HeLa cell lysate at 20 µg

Performed under reducing conditions.

Observed band size: 160 kDa



Western blot - Anti-EGFR antibody [E235] - BSA and Azide free (ab227459)

EGFR Western blot staining using rabbit Anti-EGFR antibody

False colour image of Western blot: Anti-EGFR antibody [E235] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32077 was shown to bind specifically to EGFR. A band was observed at 180 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in Egfr knockout cell line ab281597 (knockout cell lysate ab282949). To generate this image, wild-type and Egfr knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.
All lanes:
Western blot - Anti-EGFR antibody [E235] (ab32077) at 1/1000 dilution

Lane 1:
Wild-type HCT 116 cell lysate at 20 µg

Lane 2:
EGFR knockout HCT 116 cell lysate at 20 µg

Lane 2:
Western blot - Human EGFR knockout HCT116 cell line (ab281597)

Lane 3:
HeLa cell lysate at 20 µg

Lane 4:
Caco-2 cell lysate at 20 µg

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 134 kDa

Observed band size: 180 kDa

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