abcam

ab32562

Anti-EGFR antibody [E114]

Lab Essentials | Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	E114
Purification technique	Affinity purification Protein A
Specificity	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.
Epitope	ab32562 reacts with an epitope located in the C terminal region of EGF receptor.
Concentration	0.117 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Mouse
Dilution info	1/100
Notes	-

Species	Rat
Dilution info	1/100
Notes	-

Species	Human
Dilution info	1/100
Notes	-

IP

Tested

Species	Human
Dilution info	1/10 - 1/20
Notes	-

Expected

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

WB

Tested

Species	Human
Dilution info	1/1000 - 1/10000
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.

Expected

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

ICC/IF

Tested

Species	Human
Dilution info	1/10 - 1/100
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab172730 - Rabbit monoclonal IgG, 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	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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.

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.

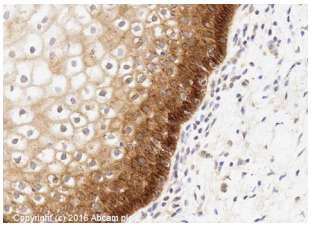
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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10 product images



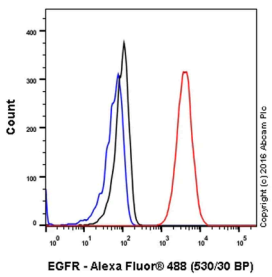
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [E114] (ab32562)

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

IHC image of EGFR staining in human nomal cervix formalin fixed paraffin embedded tissue section* performed on a Leica Bond™ system using the standard protocol F. 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 ab32562 1in50 dilution for 15 mins at room temperature and detected using an HRP conjugated compact polymer 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.

* *Tissue obtained from the Human Research Tissue Bank supported by the NIHR Cambridge Biomedical Research Centre*

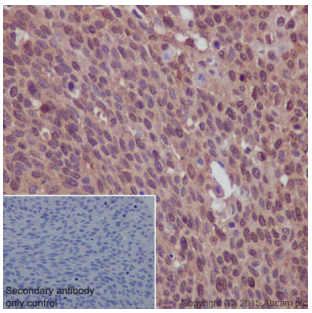


Flow Cytometry (Intracellular) - Anti-EGFR antibody [E114] (ab32562)

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

ab32562 staining EGFRin the human cell line A431 (human epidermoid carcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/20. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.
Isoytype control: Rabbit monoclonal IgG (Black)

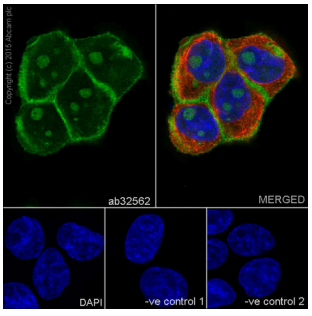
Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [E114] (ab32562)

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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labelling EGFR with purified ab32562 at a dilution of 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051 a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

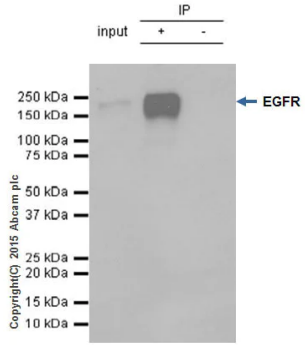


Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [E114] (ab32562)

EGFR Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-EGFR antibody

Immunocytochemistry/Immunofluorescence analysis of A431 cells labelling EGFR with purified ab32562 at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077 an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291 a mouse anti-tubulin (1/1000) and ab150120 an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.
Control 1: primary antibody (1/100) and secondary antibody ab150120 an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: ab7291 (1/1000) and secondary antibody ab150077 an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).



Immunoprecipitation - Anti-EGFR antibody [E114] (ab32562)

ab32562 (purified) at 1/20 immunoprecipitating EGFR in HeLa whole cell lysate.
Lane 1 (input): HeLa whole cell lysate (10µg)

Lane 2 (+): ab32562 + HeLa whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32562 in HeLa whole cell lysate.

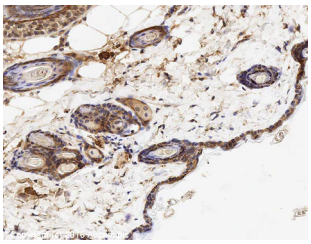
For western blotting, ab131366 VeriBlot for IP (HRP) was used for detection (1/10000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.
All lanes:
Immunoprecipitation - Anti-EGFR antibody [E114] (ab32562)

Predicted band size: 134 kDa

Observed band size: 170 kDa

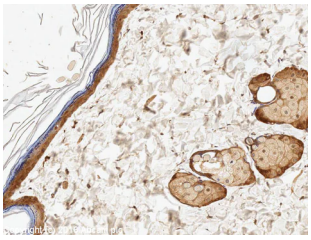


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [E114] (ab32562)

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

IHC image of EGFR staining in mouse skin formalin fixed paraffin embedded 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 ab32562 1in50 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.

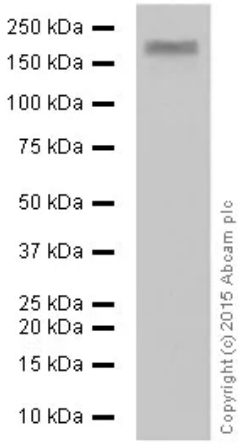


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [E114] (ab32562)

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

IHC image of EGFR staining in rat skin formalin fixed paraffin embedded tissue section performed on a Leica Bond™ system using the standard protocol F. 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 ab32562 1in50 dilution for 15 mins at room temperature and detected using an HRP conjugated compact polymer 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 [E114] (ab32562)

Blocking and dilution buffer: 5% NFDM /TBST.
All lanes:
Western blot - Anti-EGFR antibody [E114] (ab32562) at 1/10000 dilution

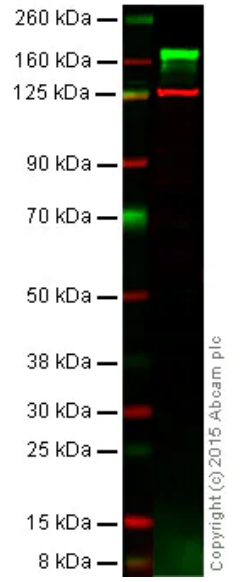
All lanes:
HeLa whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 134 kDa

Observed band size: 170 kDa



Western blot - Anti-EGFR antibody [E114] (ab32562)

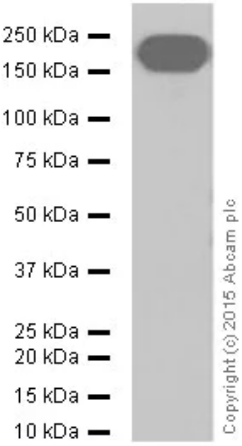
This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour before being incubated with ab32562 overnight at 4°C in the presence 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.

All lanes:
Western blot - Anti-EGFR antibody [E114] (ab32562) at 1/10000 dilution

All lanes:
A431 at 20 µg

Performed under reducing conditions.

Predicted band size: 134 kDa



Western blot - Anti-EGFR antibody [E114] (ab32562)

EGFR Western blot staining of A431 whole cell lysate using rabbit Anti-EGFR antibody

Blocking and dilution buffer: 5% NFDM /TBST.
All lanes:
Western blot - Anti-EGFR antibody [E114] (ab32562) at 1/10000 dilution

All lanes:
A431 whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 134 kDa

Observed band size: 170 kDa

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