abcam

ab32077

Anti-EGFR antibody [E235]

Anti-EGFR antibody [E235] (ab32077) is a rabbit monoclonal antibody detecting EGFR in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 30 publications
- Trusted since 2006

Lab Essentials | Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
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	0.274 - 0.291 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 - 1/200
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Expected

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

IP

Tested

Species	Human
Dilution info	1/50
Notes	-

Expected

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

WB

Tested

Species	Rat
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.

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
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/10 - 1/1000
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

What is this antibody validated in?
Anti-EGFR antibody [E235] (ab32077) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, Mouse, Rat samples.

What is the molecular weight of EGFR?
Anti-EGFR [E235] (ab32077) specifically detects a band for EGFR (UniProt: P00533) at a molecular weight of 134kDa.

Trusted by the scientific community
Anti-EGFR [E235] (ab32077) was first used in a scientific publication in 2006 and has been cited over 30 times in peer-reviewed journals.

Specificity confirmed
The specificity of Anti-EGFR antibody [E235] (ab32077) has been confirmed by Western blot testing in EGFR Knockout HeLa cell line, ab255385.

Other related products
We have a range of other formats of antibody clone [E235] also available for your convenience: ab32077, Alexa Fluor® 488 - ab205731, Alexa Fluor® 647 - ab205732, HRP - ab206036, PE - ab213332, Alexa Fluor® 555 - ab214627, Carrier free - ab227459, Biotin - ab314263, APC - ab319288, Alexa Fluor® 594 - ab319781, Alexa Fluor® 750 - ab321567

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.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size 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

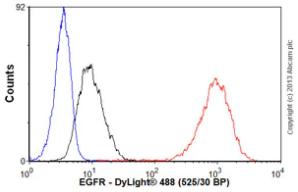
We do not recommend this combination. It is not covered by our product promise.

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.

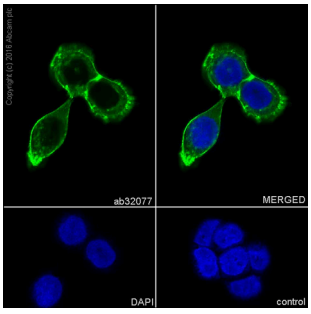
10 product images



Flow Cytometry (Intracellular) - Anti-EGFR antibody [E235] (ab32077)

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

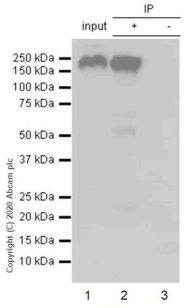
Overlay histogram showing A431 cells stained with ab32077 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions 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.



Immunocytochemistry/ Immunofluorescence - Anti-EGFR antibody [E235] (ab32077)

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.

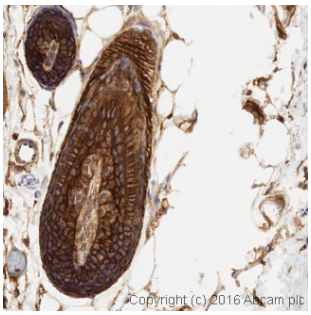


Immunoprecipitation - Anti-EGFR antibody [E235] (ab32077)

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
All lanes:

Immunoprecipitation - Anti-EGFR antibody [E235] (ab32077)

Predicted band size: 134 kDa

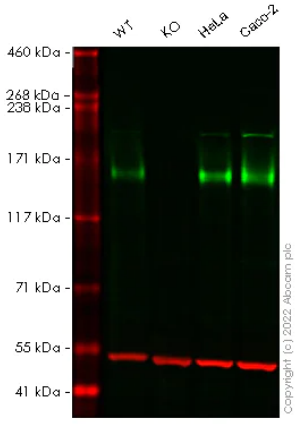


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR antibody [E235] (ab32077)

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

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] (ab32077)

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

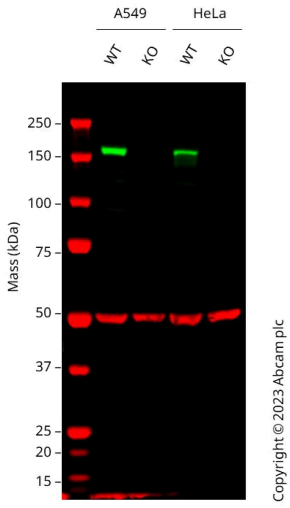
Lane 4:
Caco-2 cell lysate at 20 µg

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 134 kDa

Observed band size: 180 kDa



Western blot - Anti-EGFR antibody [E235] (ab32077)

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.

All lanes:
Western blot - Anti-EGFR antibody [E235] (ab32077) at 1/1000 dilution

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

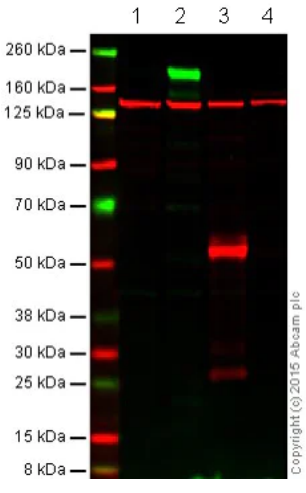
Lane 2:
EGFR knockout A549 cell lysate at 20 µg

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] (ab32077)

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 ab32077 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 [E235] (ab32077) at 1/1000 dilution

Lane 1:

Caco-2 cell lysate at 20 µg

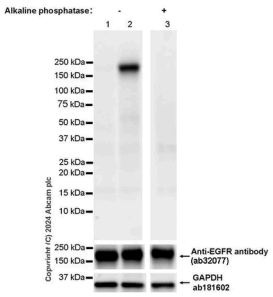
Lane 2:
A431 cell lysate at 20 µg

Lane 3:
Mouse skin cell lysate at 20 µg

Lane 4:
Rat skin cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 134 kDa



Western blot - Anti-EGFR antibody [E235] (ab32077)

EGFR Western blot staining using rabbit Anti-EGFR antibody

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-EGFR antibody - Total protein control (ab32077) staining at 1/1000 dilution.

All lanes:
Western blot - Anti-EGFR (phospho Y1045) antibody [EPR28381-88] (ab316155) at 1/1000 dilution

Lane 1:
Untreated A431 (human epidermoid carcinoma epithelial cell) whole cell lysate (untreated membrane) at 20 µg

Lane 2:
A431 treated with 100 ng/ml EGF for 30 minutes whole cell lysate (untreated membrane) at 20 µg

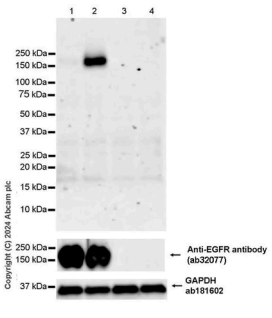
Lane 3:
A431 treated with 100 ng/ml EGF for 30 minutes whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

Secondary

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

Observed band size: 175 kDa, 36 kDa

Exposure time: 10s



Western blot - Anti-EGFR antibody [E235] (ab32077)

EGFR Western blot staining using rabbit Anti-EGFR antibody

In Western blot, ab316155 was shown to bind specifically to EGFR (phospho Y1045). Target of interest was observed at 175 kDa in wild-type HeLa cell lysates (lane 2) with no signal observed at this size in EGFR knockout cell line (lanes 3-4 KO cell line ab255385/KO cell lysate ab263845).

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-EGFR antibody (ab32077) staining at 1/1000 dilution.

All lanes:
Western blot - Anti-EGFR (phospho Y1045) antibody [EPR28381-88] (ab316155) at 1/1000 dilution

Lane 1:
Untreated Wild-type HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
Wild-type HeLa treated with 100 ng/ml EGF for 30 minutes whole cell lysate at 20 µg

Lane 3:
Untreated EGFR knockout HeLa whole cell lysate at 20 µg

Lane 4:
EGFR knockout HeLa treated with 100 ng/ml EGF for 30 minutes whole cell lysate at 20 µg

Secondary

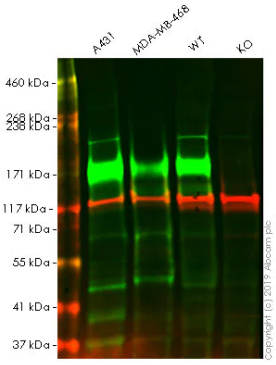
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Performed under reducing conditions.

Observed band size: 175 kDa, 36 kDa

Exposure time: 180s

Western blot - Anti-EGFR antibody [E235] (ab32077)



EGFR Western blot staining using rabbit Anti-EGFR antibody

** Lanes 1 - 4:** Merged signal (red and green). Green - ab32077 observed at 175 kDa. Red - loading control, ab130007 observed at 125 kDa. ab32077 was shown to react with EGFR in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255385 (knockout cell lysate ab263845) was used. Wild-type and EGFR knockout samples were subjected to SDS-PAGE. ab32077 and Anti-Vinculin antibody [VIN-54] (ab130007) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.
All lanes:
Western blot - Anti-EGFR antibody [E235] (ab32077) at 1/1000 dilution

Lane 1:
A431 cell lysate at 20 µg

Lane 2:
MDA-MB-468 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

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Predicted band size: 134 kDa

Observed band size: 124 kDa, 134 kDa

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