abcam

Product datasheet

Anti-EGFR (phospho Y845) antibody [EPR2149Y] ab97613

Recombinant RabMAb

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Overview

Product name	Anti-EGFR (phospho Y845) antibody [EPR2149Y]
Description	Rabbit monoclonal [EPR2149Y] to EGFR (phospho Y845)
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), Dot blot, WB, ICC/IF **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human EGFR (phospho S845). The exact sequence is proprietary.
Positive control	A431 cells and cell lysates.
General notes	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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR2149Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab97613 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)		1/400.
Dot blot		Use at an assay dependent concentration.
WB		1/500. Detects a band of approximately 175 kDa (predicted molecular weight: 134 kDa).
ICC/IF		1/100 - 1/250.

Application notes Is unsuitable for IHC-P.

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.

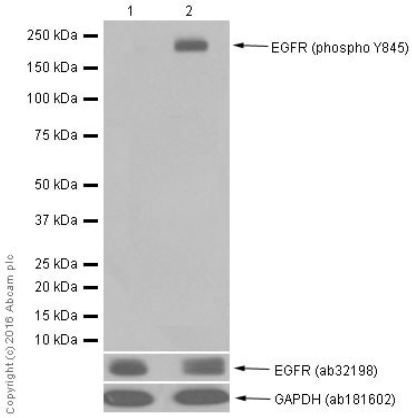
Involvement in disease Lung cancer
Inflammatory skin and bowel disease, neonatal, 2

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



Western blot - Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613)

All lanes : Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613) at 1/1500 dilution (purified)

Lane 1 : Untreated A431 (Human epidermoid carcinoma cell line) whole cell lysates
Lane 2 : A431 (Human epidermoid carcinoma cell line) treated with 100 ng/ml EGF for 10 minutes whole cell lysates

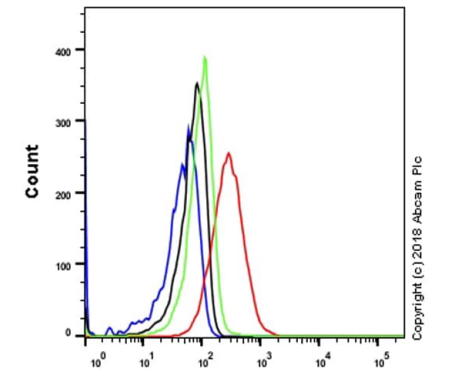
Lysates/proteins at 15 µg per lane.

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

Predicted band size: 134 kDa
Observed band size: 175 kDa

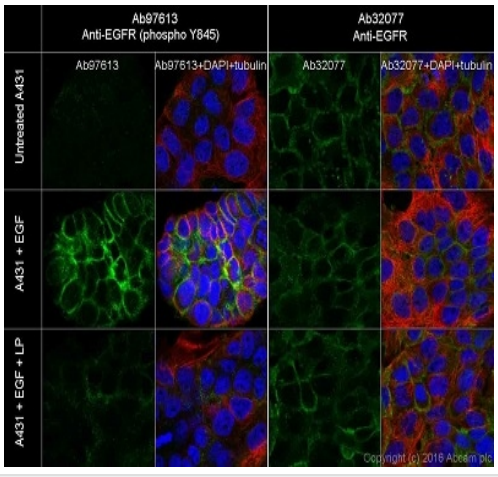
Exposure time: 30 seconds

Blocking and dilution buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613)

Intracellular Flow Cytometry analysis of A431 (Human epidermoid carcinoma epithelial cell) treated with 100ng/ml EGF for 10 minutes cells labeling EGFR with purified ab97613 at 1/400 dilution (1µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr® 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).



Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613)

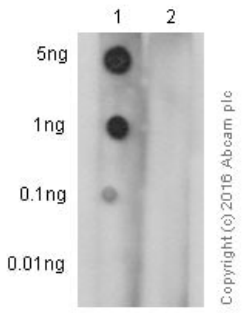
Immunofluorescent analysis of 4% paraformaldehyde-fixed,0.1% Triton X-100 permeabilized A431 (Human epidermoid carcinoma cell line) cell lines labeling EGFR with ab97613 (purified) at 1/50 dilution,followed by Goat anti-Rabbit IgG H & L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution

Cells were counterstained with **ab195889** (Anti-alpha Tubulin antibody [DM1A]) - Microtubule Marker (Alexa Fluor® 594)

The nuclear counterstain is DAPI (blue).

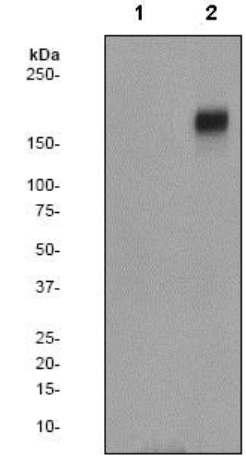
The green staining on the membrane was increased in the EGF (100ng/ml, 10min) treated A431 cells when compared with A431 cells without treatment. After LP treatment, the green signaling was obviously decreased.

For the pan antibody, there was no great difference after EGF (100ng/ml, 10min) or EGF (100ng/ml, 10min) + LP treatment. The data showed mostly membranous staining.



Dot Blot - Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613)

Dot Blot analysis of Lane 1: EGFR (pY845) phospho peptide and Lane 2: EGFR non-phospho peptide labeling EGFR (phospho Y845) with ab97613 (unpurified) at 1/1000 dilution. 5% NFDM/TBST was used as the diluting and blocking buffer. **ab97051** Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated was used as the secondary antibody at 1/100000 dilution. Exposure time: 3 minutes.



Western blot - Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613)

All lanes : Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613) at 1/500 dilution (unpurified)

Lane 1 : A431 cell lysates, untreated
Lane 2 : A431 cell lysates, treated with EGF

Lysates/proteins at 10 µg per lane.

Predicted band size: 134 kDa

Anti-EGFR (phospho Y845) antibody [EPR2149Y] (ab97613)

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