abcam

Product datasheet

Human EGFR (pY1068) + total EGFR ELISA Kit ab126439

3 Images

Overview

Product name	Human EGFR (pY1068) + total EGFR ELISA Kit
Detection method	Colorimetric
Sample type	Cell Lysate
Assay type	Semi-quantitative
Assay time	5h 00m
Assay duration	Multiple steps standard assay
Species reactivity	**Reacts with:** Human
Product overview	ab126439 is a very rapid, convenient and sensitive assay kit that can monitor the activation or function of important biological pathways in cell lysates. By determining phosphorylated EGFR protein in your experimental model system, you can verify pathway activation in your cell lysates. You can simultaneously measure numerous different cell lysates without spending excess time and effort in performing a Western Blot analysis. This Sandwich ELISA kit is an in vitro enzyme-linked immunosorbent assay for the measurement of human phospho-EGFR (Tyr 1068) and total EGFR (help normalize the results of phospho-EGFR from different cell lysate being compared). An anti-EGFR antibody has been coated onto a 96-well plate. Samples are pipetted into the wells and phosphorylated and total EGFR present in a sample is bound to the wells by the immobilized antibody. The wells are washed and anti-phosphorylated EGFR (Tyr 1068) or anti-total-EGFR antibody is used to detect phosphorylated or non-phosphorylated EGFR. After washing away unbound antibody, HRP-conjugated anti-Rabbit IgG or HRP-Streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of EGFR (Tyr 1068) or total EGFR bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.
Notes	Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.
Platform	Microplate

Properties

Storage instructions Store at -20°C. Please refer to protocols.

Components	1 x 96 tests
20X Wash Buffer	1 x 25ml
2X Cell Lysis Buffer	1 x 5ml
500X HRP-conjugated anti-rabbit IgG	1 x 25µl
5X Assay Diluent	1 x 15ml
600X HRP-Streptavidin Concentrate	1 x 200µl
Anti-phospho EGFR (Y1068)	1 vial
EGFR Microplate (12 strips x 8 wells) coated with monoclonal anti-EGFR antibody	1 unit
Pan Detection Antibody EGFR: Goat anti-pan EGFR	1 vial
Positive Control: lyophilized powder from A431 cell lysate	1 vial
Stop Solution	1 x 8ml
TMB One-Step Substrate Reagent	1 x 12ml

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.

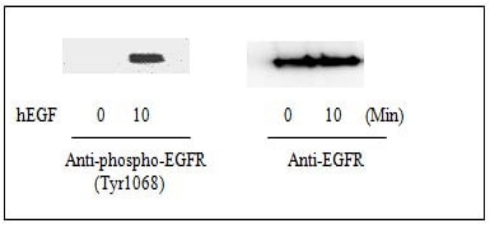
Involvement in disease Lung cancer
Inflammatory skin and bowel disease, neonatal, 2

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

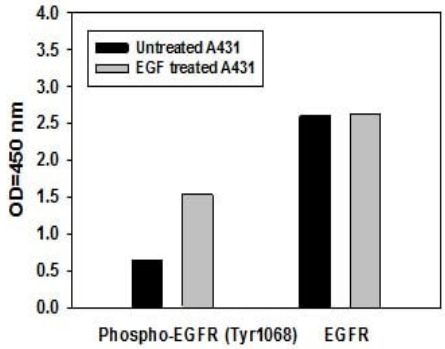
	linkage is mainly through 'Lys-63', but linkage through 'Lys-48', 'Lys-11' and 'Lys-29' also occurs. Deubiquitination by OTUD7B prevents degradation. Ubiquitinated by RNF115 and RNF126. Methylated. Methylation at Arg-1199 by PRMT5 stimulates phosphorylation at Tyr-1197.
Cellular localization	Secreted 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



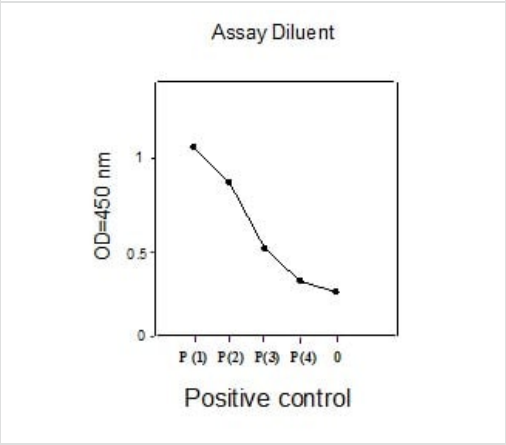
Western blot - EGFR (pY1068) + total EGFR Human ELISA Kit (ab126439)

A431 cells were treated or untreated with 100 ng/ml recombinant human EGF for 10 min. Cell lysates were analyzed by Western Blot.



Sandwich ELISA - EGFR (pY1068) + total EGFR Human ELISA Kit (ab126439)

A431 cells were treated or untreated with 100 ng/ml recombinant human EGF for 10 min. Cell lysates were analyzed using ab126439.



Sandwich ELISA - EGFR (pY1068) + total EGFR Human ELISA Kit (ab126439)

A431 cells were treated with recombinant human EGF at 37°C for 10 min. Solubilize cells at 4 x 10^7 cells/ml in Cell Lysate Buffer. Serial dilutions of lysates were analyzed using ab126439.

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