abcam

Product datasheet

Human EGFR (Tyr1068) In-Cell ELISA Kit ab126420

2 Images

Overview

Product name	Human EGFR (Tyr1068) In-Cell ELISA Kit
Detection method	Colorimetric
Sample type	Adherent cells
Assay type	Cell-based (qualitative)
Assay time	5h 10m
Assay duration	Multiple steps standard assay
Species reactivity	**Reacts with:** Human
Product overview	ab126420 is a very rapid, convenient and sensitive assay kit that can monitor the activation or function of important biological pathways in cells. It can be used for measuring the relative amount of EGFR (Tyr1068) phosphorylation and screening the effects of various treatments, inhibitors (such as siRNA or chemicals), or activators in cultured Human cell lines. By determining EGFR protein phosphorylation in your experimental model system, you can verify pathway activation in your cell lines without spending excess time and effort in preparing cell lysate and performing an analysis of Western Blot. In the EGFR (Tyr1068) Human In-Cell ELISA Kit, cells are seeded into a 96 well tissue culture plate. The cells are fixed after various treatments, inhibitors or activators. After blocking, anti-Phospho-EGFR (Tyr1068) or anti-EGFR (primary antibody) is pipetted into the wells and incubated. The wells are washed, and HRP-conjugated anti-rabbit IgG (secondary antibody) is added to the wells. The wells are washed again, a TMB substrate solution is added to the wells and color develops in proportion to the amount of protein. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.
Platform	Microplate

Properties

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

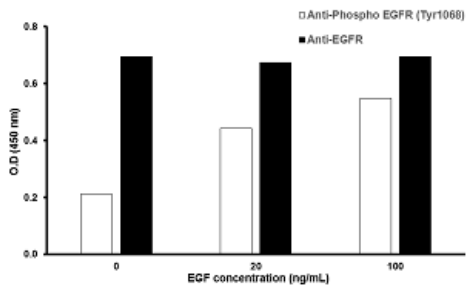
Components	1 x 96 tests
Anti-Rabbit IgG Concentrate (Item I-1)	1 x 10µl

Components	1 x 96 tests
Blocking Buffer Concentrate (5X)	1 x 20ml
Fixing Solution	1 x 30ml
Uncoated 96-well Microplate	1 unit
Quenching Buffer Concentrate (30x)	1 x 2ml
Rabbit Anti-EGFR Concentrate (Item H)	1 x 6µl
Rabbit Anti-Phospho-EGFR (Tyr1068) Concentrate (Item G)	1 x 6µl
Stop Solution	1 x 14ml
TMB One-Step Substrate Reagent	1 x 12ml
Wash Buffer A Concentrate (20X)	1 x 30ml
Wash Buffer B Concentrate (20X)	1 x 30ml

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.

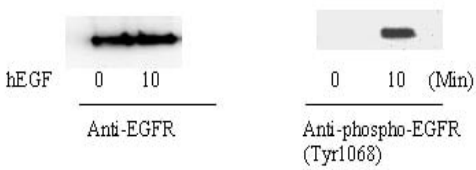
Cellular localization	Secreted and Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus membrane. Nucleus membrane. Endosome. Endosome membrane. Nucleus. In response to EGF, translocated from the cell membrane to the nucleus via Golgi and ER. Endocytosed upon activation by ligand. Colocalized with GPER1 in the nucleus of estrogen agonist-induced cancer-associated fibroblasts (CAF).

Images



In-Cell ELISA - EGFR (Tyr1068) Human In-Cell ELISA Kit (ab126420)

A431 cells were stimulated by different concentrations of EGF for 10 min at 37°C.



Western blot - EGFR (Tyr1068) Human In-Cell ELISA Kit (ab126420)

Western blot analysis of extracts from 100 ng/ml hEGF treated A431 cells. Phospho-EGFR (Tyr1068) and EGFR antibodies were used in both detection assays.

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