abcam

Product datasheet

Human EGFR In-Cell ELISA Kit ab126419

3 References 2 图像

概述

产品概述

产品名称 人EGFR In-Cell ELISA试剂盒

检**测方法** Colorimetric 样**品**类型 Adherent cells

检测类型 Cell-based (qualitative)

检测时间 5h 10m

实验步骤 Multiple steps standard assay

种属反应性 与反应: Human

ab126419 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 (activated) 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 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 (activated) or Anti-EGFR (primary antibody) is pipetted into the wells and incubated. The wells are washed, and HRP-conjugated anti mouse 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.

平台 Microplate

性能

存放说明 Store at -20°C. Please refer to protocols.

组 件	1 x 96 tests
HRP-conjugated Anti-Mouse IgG Concentrate	1 x 10µl
Blocking Buffer Concentrate (5X)	1 x 20ml

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组 件	1 x 96 tests
Fixing Solution	1 x 30ml
Uncoated 96-well Microplate	1 unit
Mouse Anti-EGFR Concentrate (Item H)	1 x 7µI
Mouse Anti-Phospho-EGFR (active) Concentrate (Item G)	1 x 7µI
Quenching Buffer Concentrate (30x)	1 x 2ml
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

功能

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.

Ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.

组织特异性

Lung cancer

疾病相关

Inflammatory skin and bowel disease, neonatal, 2

序列相似性

Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 1 protein kinase domain.

翻译后修饰

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.

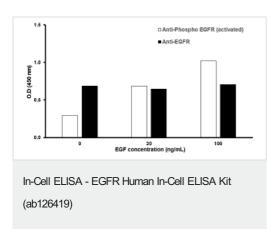
Monoubiquitinated and polyubiquitinated upon EGF stimulation; which does not affect tyrosine kinase activity or signaling capacity but may play a role in lysosomal targeting. Polyubiquitin linkage is mainly through 'Lys-63', but linkage through 'Lys-48', 'Lys-11' and 'Lys-29' also occurs. Deubiquitination by OTUD7B prevents degradation. Ubiquitinated by RNF115 and RNF126. Methylated. Methylation at Arg-1199 by PRMT5 stimulates phosphorylation at Tyr-1197.

细胞定位

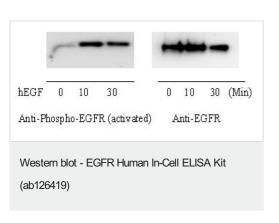
Secreted and Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus membrane. Nucleus membrane. Endosome. Endosome membrane. Nucleus. In response to EGF,

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).

图片



A431 cells were stimulated by different concentrations of EGF for 20 min at 37°C.



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

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