abcam

Product datasheet

Anti-EGFR (phospho Y1068) antibody ab5644

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概述

产品名称	Anti-EGFR (phospho Y1068)**抗体**
描述	**兔多克隆抗体**to EGFR (phospho Y1068)
宿主	Rabbit
经测试应用	**适用于:** ICC/IF, WB
种属反应性	**与反应:** Human
免疫原	Synthetic peptide corresponding to Human EGFR (phospho W1068).
阳性对照	WB: A-431 and A549 whole cell lysate treated with EGF (200 ng/mL for 10 minutes). ICC: A-431 cells treated with 200 ng/mL of EGF for 10 minutes.
常规说明	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
存储溶液	pH: 7.30 Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol, 0.1% BSA
纯度	Immunogen affinity purified
纯化说明	The antibody has been negatively preadsorbed using (i) a non phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated epidermal growth factor receptor (EGFR), and (ii) a generic tyrosine phosphorylated peptide to remove antibody that is reactive with phosphotyrosine, irrespective of the sequence. The final product is generated by affinity chromatography using an EGFR-derived peptide that is phosphorylated at tyrosine 1068.
克隆	**多克隆**

同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab5644于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

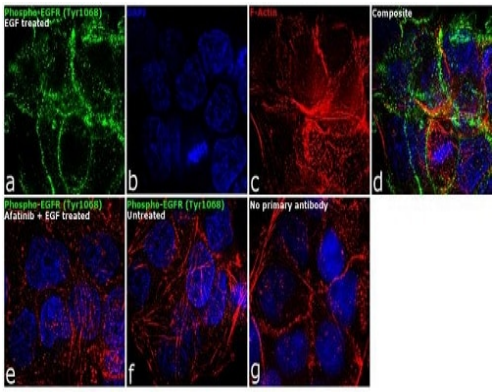
应用	Ab评论	说明
ICC/IF		1/100.
WB	★★★★☆ (5)	1/1000. Detects a band of approximately 185 kDa.

靶标

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.
组织特异性	Ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.
疾病相关	Lung cancer 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).

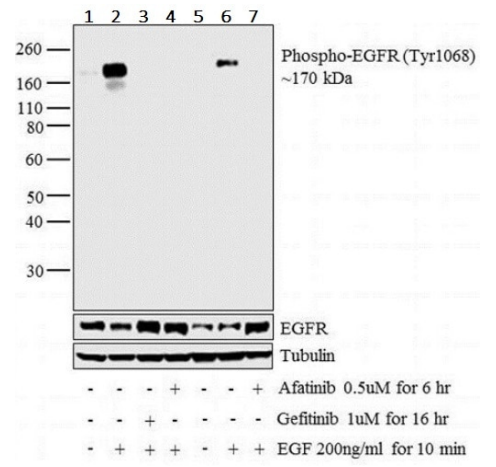
图片



Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho Y1068) antibody (ab5644)

Immunofluorescence analysis of A431 (Human epidermoid carcinoma cell line) cells labeling EGFR with ab5644 at 1/100 dilution, followed by Goat anti-rabbit IgG (H+L) Superclonal, Alexa Fluor® 488 conjugate was used as the secondary antibody at 1/2000 dilution (Panel a). Nuclei (Panel b) were stained with SlowFade® Gold Antifade Mountant with DAPI. F-actin (Panel c) was stained with Rhodamine Phalloidin. Panel (d) represents the merged image showing membrane localization. Panel (e) represents cells treated with antagonist, Afatinib (1µM for 6hrs) followed by EGF (200 ng/ml for 10 minutes), showing no Phospho-EGFR staining. Panel (f) shows untreated cells with no signal. Panel (g) represents control cells with no primary antibody to assess background.

The cells (in 70% confluent log phase treated with 200ng/ml of EGF for 10 minutes) were fixed with 4% paraformaldehyde for 10 minutes; permeabilized with 0.1% Triton X-100 for 10 minutes and blocked with 1% BSA for hour at room temperature. The images were captured at 60X magnification.



Western blot - Anti-EGFR (phospho Y1068) antibody (ab5644)

All lanes : Anti-EGFR (phospho Y1068) antibody (ab5644) at 1/1000 dilution

Lane 1 : A431 (Human epidermoid carcinoma cell line) whole cell lysate with skimmed milk

Lane 2 : A431 whole cell lysate treated with EGF (200 ng/mL for 10 minutes) with skimmed milk

Lane 3 : A431 whole cell lysate treated with Gefitinib followed by EGF (1uM for 16 hours, 200 ng/mL for 10 minutes) with skimmed milk

Lane 4 : A431 whole cell lysate treated with Afatinib followed by EGF (0.5 uM for 6 hours, 200 ng/mL for 10 minutes) with skimmed milk

Lane 5 : A549 (Human lung carcinoma cell line) whole cell lysate with skimmed milk

Lane 6 : A549 whole cell lysate treated with EGF (200 ng/mL for 10 minutes) with skimmed milk

Lane 7 : A549 whole cell lysate treated with Afatinib followed by EGF (0.5 uM for 6 hours, 200 ng/mL for 10 minutes) with skimmed

milk

Lysates/proteins at 30 µg per lane.

Blocking peptides at 5 % per lane.

Secondary
All lanes : Goat anti-Rabbit IgG Superclonal Secondary Antibody, HRP at 1/4000 dilution

Western blot analysis using ab5644 shows increased expression of proteins phosphorylated at the tyrosine residues in A-431 and A549 cell lines upon EGF treatment and pre-treatment with EGFR-antagonists, Gefitinib and Afatinib. This results in inhibition of Phospho-EGFR in A-431 and A549 cell lines

Western blot - Anti-EGFR (phospho Y1068) antibody (ab5644)

This image is courtesy of an Abreview submitted by Mr Samir Nuseibeh

All lanes : Anti-EGFR (phospho Y1068) antibody (ab5644) at 1/200 dilution

Lane 1 : A431 (Human epidermoid carcinoma cell line) whole cell lysate - not treated
Lane 2 : A431 whole cell lysate - 100ng/ml EGF for 1 minute
Lane 3 : A431 whole cell lysate - 100ng/ml EGF for 2.5 minutes
Lane 4 : A431 whole cell lysate - 100ng/ml EGF for 5 minutes
Lane 5 : A431 whole cell lysate - 100ng/ml EGF for 10 minutes
Lane 6 : A431 whole cell lysate - 100ng/ml EGF for 20 minutes
Lane 7 : A431 whole cell lysate - 100ng/ml EGF for 40 minutes

Secondary
All lanes : HRP conjugated Goat anti-rabbit antibody

Developed using the ECL technique.

Performed under reducing conditions.

Exposure time: 2 minutes

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