abcam

Product datasheet

Anti-EGFR (phospho T669) antibody [EP2256Y] ab75980

重组 RabMAb

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概述

产品名称 Anti-EGFR (phospho T669)抗体[EP2256Y]

描述 兔单克隆抗体[EP2256Y] to EGFR (phospho T669)

宿主 Rabbit

特异性 Recognises EGFR phosphorylated on Threonine 669 of the mature human isoform 1

(corresponding to T693 from the precursor form P00533-1/p170)

适用于: ICC/IF, Dot blot, WB 经测试应用

不适用于: Flow Cyt or IHC-P

种属反应性 与反应: Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 WB: A431 treated with 100ng/ml Epidermal Growth Factor (EGF). ICC/IF: A431 cells. Dot Blot:

EGFR (phospho T669) peptide.

常规说明 This product has switched from a hybridoma to recombinant production method on 9th February

2024.

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.

性能

形式

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: 7.20

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS

纯度 Protein A purified

同种型 IgG

应用

The Abpromise guarantee

Abpromise™承诺保证使用ab75980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		1/500.
Dot blot		1/1000.
WB		1/2000. Detects a band of approximately 175 kDa (predicted molecular weight: 134 kDa).

应用说明 Is unsuitable for Flow Cyt or IHC-P.

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/betacatenin.

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

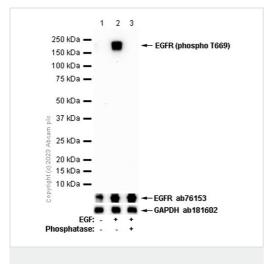
STAT3. Dephosphorylated by PTPN1 and PTPN2.

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



Western blot - Anti-EGFR (phospho T669) antibody [EP2256Y] (ab75980) **All lanes :** Anti-EGFR (phospho T669) antibody [EP2256Y] (ab75980) at 1/2000 dilution

Lane 1 : Untreated A431 (Human epidermoid carcinoma) whole cell lysate

Lane 2: A431 treated with 100ng/ml Epidermal Growth Factor (EGF) for 30mins whole cell lysate

Lane 3: A431 treated with 100ng/ml Epidermal Growth Factor (EGF) for 30mins whole cell lysate, then the membrane treated with Alkaline Phosphatase for 1 hour

Lysates/proteins at 15 µg per lane.

Secondary

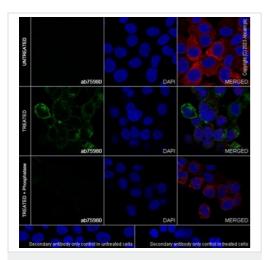
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Performed under reducing conditions.

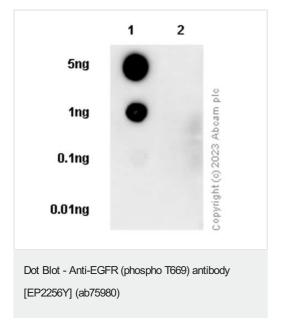
Predicted band size: 134 kDa **Observed band size:** 170 kDa

Exposure time: 40 seconds

Blocking and diluting buffer and concentration: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho T669) antibody [EP2256Y] (ab75980) Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized A431 cells labelling EGFR with ab75980 at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) at 1/1000 dilution. Alexa Fluor® 594 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (ab195889) was used to counterstain tubulin at 1/200 dilution. The nuclear counterstain was DAPI (Blue).



Dot blot analysis of EGFR (phospho T669) using ab75980 at 1/1000 dilution, followed by a Goat Anti-Rabbit lgG (H+L) Peroxidase conjugated (ab97051) at 1/2500 dilution. Blocking and dilution buffer: 5% NFDM/TBST. Exposure time: 41s.

Lane 1: EGFR (phospho T669) peptide

Lane 2: EGFR non-phospho peptide

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