abcam

Product datasheet

Anti-EGFR (phospho Y1068) antibody [EP774Y] ab40815

重组 RabMAb

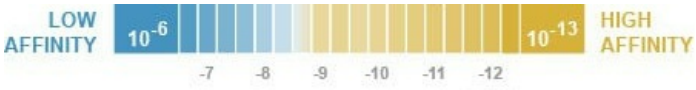
14 Abreviews 130 References 17 图像

概述

产品名称	Anti-EGFR (phospho Y1068)抗体[EP774Y]
描述	兔单克隆抗体[EP774Y] to EGFR (phospho Y1068)
宿主	Rabbit
特异性	Recognises EGFR phosphorylated on Tyrosine 1068 of the mature human isoform 1 (corresponding to Y1092 from the precursor form P00533-1/p170) The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse.
经测试应用	**适用于:** Flow Cyt (Intra), Dot blot, WB, ICC/IF, IHC-P
种属反应性	**与反应:** Mouse, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	IHC-P: Human breast adenocarcinoma, papillary carcinoma of thyroid glad, glioma, cervical carcinoma and prostate cancer tissue; Mouse E17 embryo head tissue and mouse pancreas tissue. ICC/IF: A431 cells and WD-PBEC cultures. WB: A431 cells treated with EGF. Dot Blot: EGFR (pY1068) peptide.
常规说明	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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
解离常数 (K_D)	K_D = 3.60 x 10 $^{-11}$ M



Learn more about K_D

存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EP774Y
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab40815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。

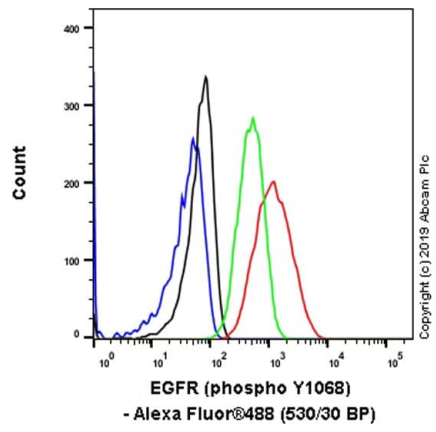
应用	Ab评论	说明
Flow Cyt (Intra)		1/800.
Dot blot		1/1000.
WB	★★★★☆ (2)	1/500 - 1/5000. Predicted molecular weight: 170 kDa.
ICC/IF		1/250 - 1/500.
IHC-P	★★★★★ (11)	1/250 - 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse.

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.

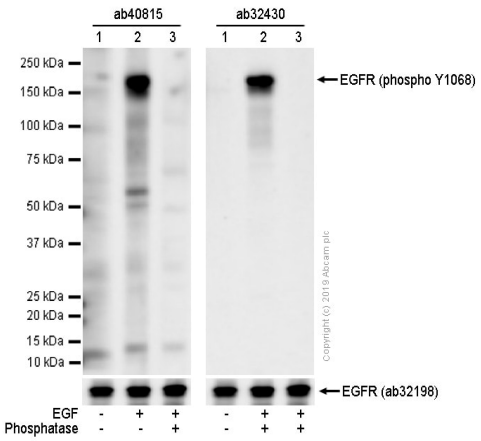
	Isoform 2 may act as an antagonist of EGF action.
组织特异性	Ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.
疾病相关	Lung cancer Inflammatory skin and bowel disease, neonatal, 2
序列相似性	Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 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



Flow Cytometry (Intracellular) - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

Intracellular Flow Cytometry analysis of A431 (Human epidermoid carcinoma epithelial cell) treated with 200 ng/ml EGF for 15 minutes cells labeling EGFR with purified ab40815 at 1/800 dilution (1 µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue). Untreated A431 cells (Green).



Western blot - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

All lanes : Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815) at 1/1000 dilution (Purified)

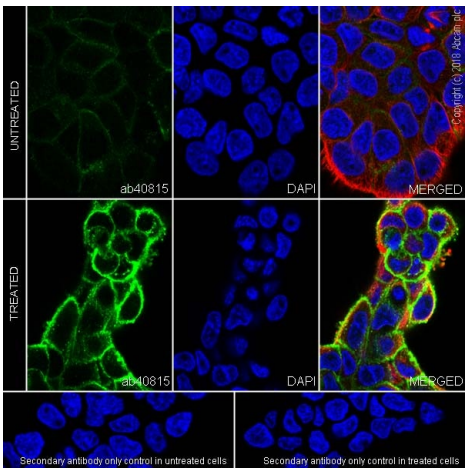
Lane 1 : A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates
Lane 2 : A431 (Human epidermoid carcinoma epithelial cell) treated with 10 ng/ml EGF for 30 minutes whole cell lysates
Lane 3 : A431 (Human epidermoid carcinoma epithelial cell) treated with 10 ng/ml EGF for 30 minutes. Then the membrane was incubated with phosphatase

Lysates/proteins at 15 µg per lane.

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

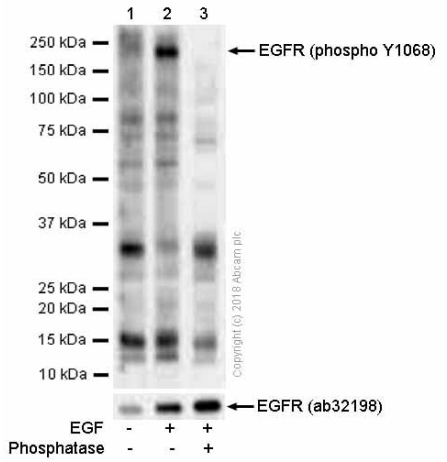
Predicted band size: 170 kDa
Observed band size: 175 kDa

This antibody detects high background.



Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

Immunocytochemistry/ Immunofluorescence analysis of A431 (Human epidermoid carcinoma epithelial cell) treated with 100 ng/ml EGF for 10 minutes cells labeling EGFR with purified ab40815 at 1:500 dilution (1.8 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

All lanes : Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815) at 1/1000 dilution (purified)

Lane 1 : A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates
Lane 2 : A431 (Human epidermoid carcinoma epithelial cell) treated with 10 ng/ml EGF for 30 minutes whole cell lysates
Lane 3 : A431 (Human epidermoid carcinoma epithelial cell) treated with 10 ng/ml EGF for 30 minutes. Then the membrane was incubated with phosphatase

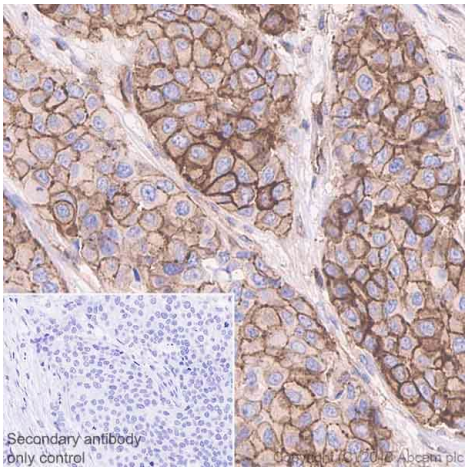
Lysates/proteins at 15 µg per lane.

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

Predicted band size: 170 kDa
Observed band size: 175 kDa

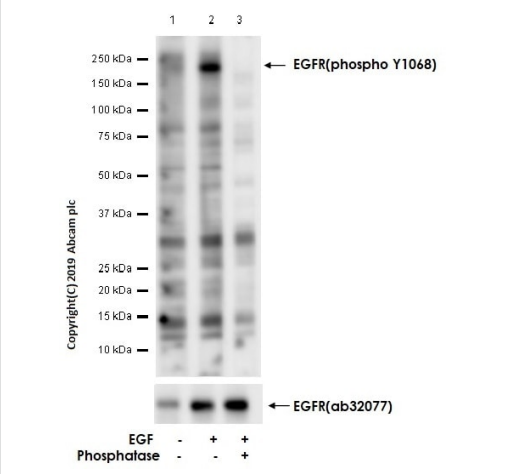
Exposure time: 30 seconds

This antibody detects high background.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast cancer tissue sections labeling EGFR with purified ab40815 at 1:500 dilution (1.75 µg/ml). Heat mediated antigen retrieval was performed using heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

All lanes : Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815) at 1/1000 dilution

Lane 1 : C2C12 (Mouse myoblasts myoblast) whole cell lysate
Lane 2 : C2C12 (Mouse myoblasts myoblast) treated with 100 ng/ml EGF for 24 hours whole cell lysate
Lane 3 : C2C12 (Mouse myoblasts myoblast) treated with 100 ng/ml EGF for 24 hours whole cell lysate. Then the membrane was incubated with phosphatase.

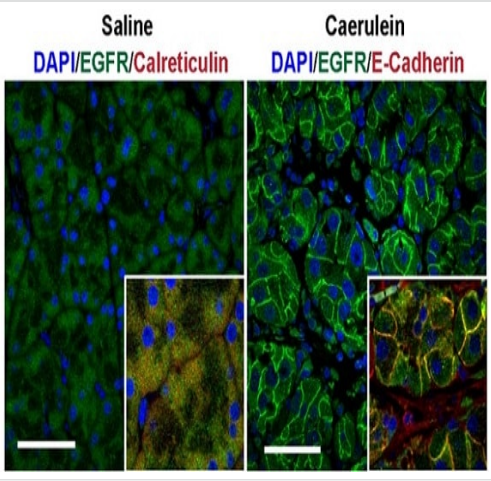
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 170 kDa
Observed band size: 175 kDa

Exposure time: 30 seconds

Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

Wodziak D et al., PLoS One, 11, e0164968, 2016
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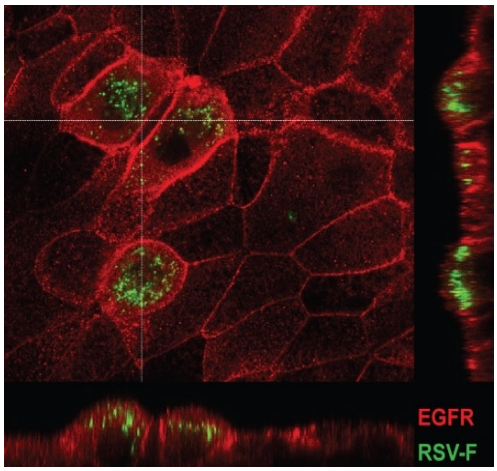
Total EGFR (green) subcellular localization with and without caerulein-induced pancreatitis as determined by immunohistochemistry and confocal imaging. The nuclei were identified with DAPI stain (blue). Scale bars = 50 µm. Calreticulin and E-cadherin (red) served as markers for the endoplasmic reticulum and plasma membrane, respectively, and were used to quantify EGFR subcellular location. Pancreatitis was induced with the 1-day protocol of 8 hourly caerulein injections in 6–8 week old wild-type (wt) and 3-week old $AGR2^{-/-}$ (ko) mice.

For immunofluorescence, antigen retrieval was performed in a pressure cooker set to 118°C. The slides were incubated in antigen unmasking solution (DAKO) for 3 min followed by equilibration at room temperature for 1 hr. The slides were then placed in 5% serum blocking solution (goat, horse, or rabbit serum as appropriate) for 30 min to block nonspecific binding of antibody to the tissue. The sections were incubated with primary antibody diluted in 2% serum overnight at 4°C. The respective secondary

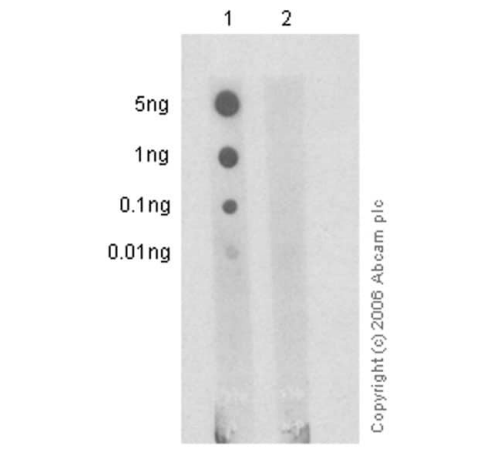
antibodies were used at predetermined dilutions. Immunofluorescence slides were mounted with media containing DAPI stain (Vectashield, Vector Laboratories).



Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

WD-PBEC cultures were infected with RSV clinical isolate BT2a and stained for EGFR (red) and RSV F (RSV F, green) expression.

For WD-PBECs, pediatric bronchial epithelial cells (PBEC) were obtained, via written parental consent, from bronchial brushings of children undergoing elective surgery at the Royal Belfast Hospital for Sick Children, and the procedures were approved by the Office of Research Ethics Committees Northern Ireland. PBEC were expanded in collagen-coated flasks using airway epithelial cell media and supplements (Lonza), then seeded onto transwell inserts (Corning), and then air-liquid interface (ALI) cultures were initiated and maintained 21 days in order to establish well-differentiated (WD)-PBECs. Paraformaldehyde-fixed and permeabilized WD-PBEC were stained for RSV F protein expression and were stained with anti-phospho-(p)-EGFR (Abcam, ab40815, unpurified). WD-PBEC cultures were infected with RSV subgroup A clinical isolate BT2a. Fluorescent images were obtained with a SP5 confocal DMI 6000 inverted microscope (Leica).

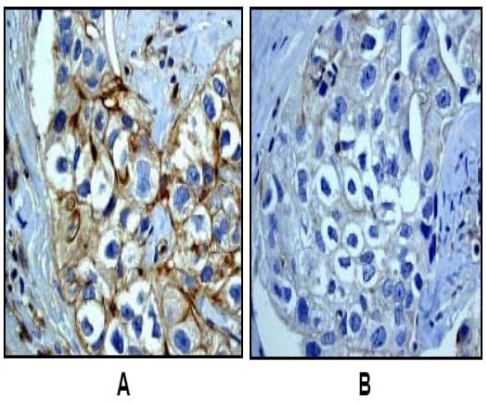


Dot Blot - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

Dot blot analysis of EGFR (pY1068) peptide (Lane 1), SMAD5 (unmodified) peptide labelling EGFR (pY1068) with ab40815 (unpurified) at a dilution of 1/1000. Peroxidase conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody at a dilution of 1/2500.

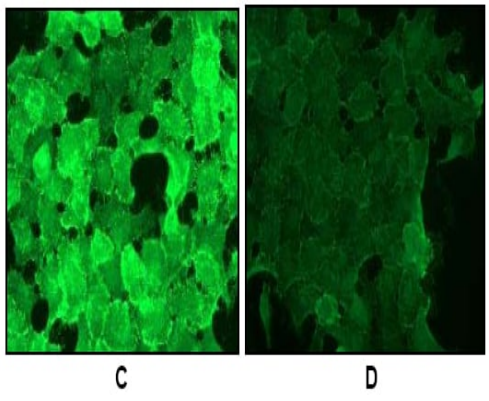
Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



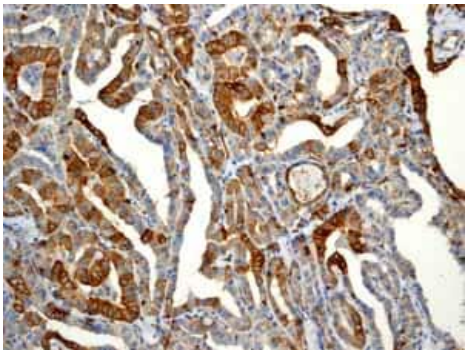
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

Immunohistochemical staining of untreated (A) and Phosphatase-treated (B) paraffin-embedded breast adenocarcinoma tissue using ab40815 (unpurified).



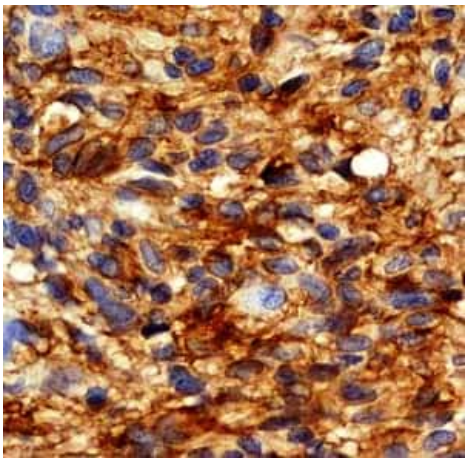
Immunocytochemistry/ Immunofluorescence - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

Immunofluorescent staining of untreated (C) and Phosphatase-treated (D) A431 cells using ab40815 (unpurified).



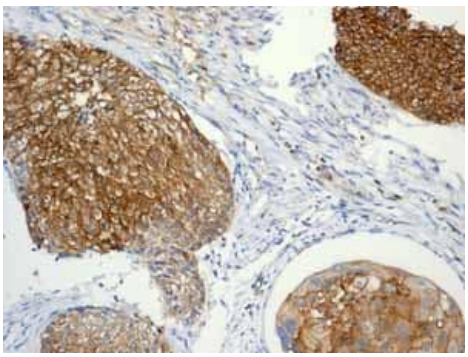
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

ab40815 (unpurified) showing positive staining in Papillary carcinoma of thyroid gland tissue.



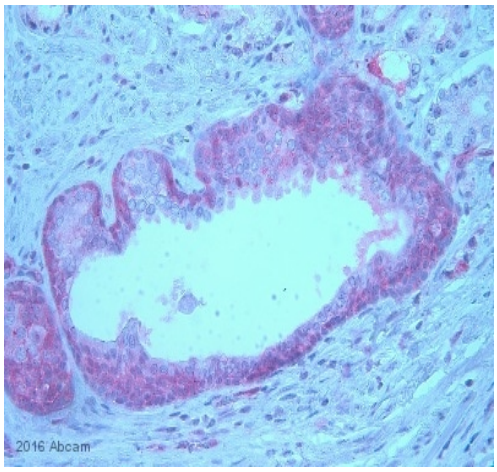
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

ab40815 (unpurified) showing positive staining in Glioma tissue.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

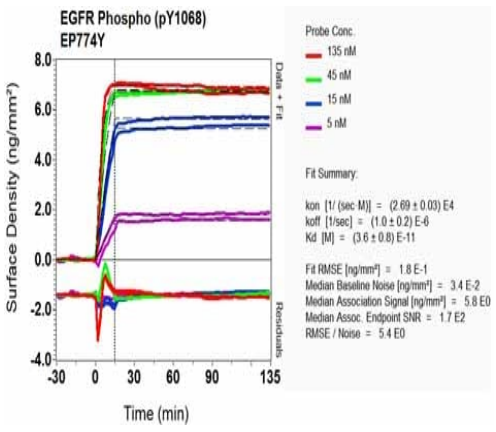
ab40815 (unpurified) showing positive staining in Cervical carcinoma tissue.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

This image is submitted courtesy of an anonymous Abreview.

Formaldehyde-fixed, paraffin-embedded human prostate cancer tissue stained for EGFR (phospho Y1068) using ab40815 (unpurified) at 1/200 dilution in immunohistochemical analysis, followed by Goat anti Rabbit IgG (Biotin).

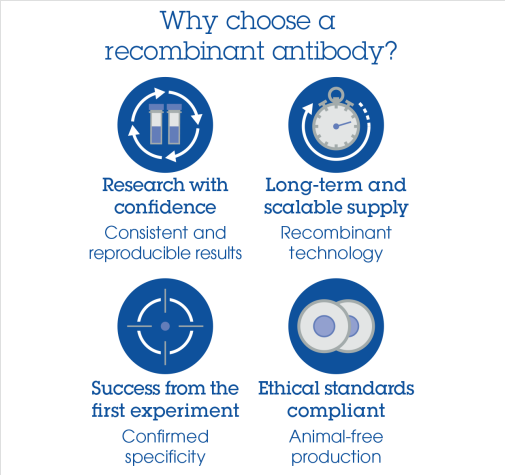


OI-RD Scanning - Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D



Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

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