

# Alexa Fluor® 647 Anti-EGFR (phospho Y1068) antibody [EP774Y] $\alpha$ b205828

Recombinant RabMAb

6 Images

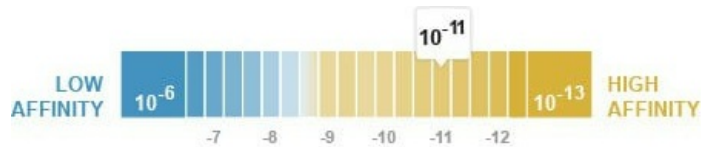
## Overview

<b>Product name</b>	Alexa Fluor® 647 Anti-EGFR (phospho Y1068) antibody [EP774Y]
<b>Description</b>	Alexa Fluor® 647 Rabbit monoclonal [EP774Y] to EGFR (phospho Y1068)
<b>Host species</b>	Rabbit
<b>Conjugation</b>	Alexa Fluor® 647. Ex: 652nm, Em: 668nm
<b>Specificity</b>	Recognises EGFR phosphorylated on Tyrosine 1068 of the mature human isoform 1 (corresponding to Y1092 from the precursor form P00533-1/p170)
<b>Tested applications</b>	<b>Suitable for:</b> ICC/IF, Flow Cyt (Intra), IHC-P
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Human
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	ICC/IF: A431 cells treated with EGF (100ng/ml, 5min), C2C12 cells. IHC-P: Human breast tissue and human breast cancer tissue
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb® patents</a>.</p> <p>Alexa Fluor® is a registered trademark of Molecular Probes, Inc, a Thermo Fisher Scientific Company. The Alexa Fluor® dye included in this product is provided under an intellectual property license from Life Technologies Corporation. As this product contains the Alexa Fluor® dye, the purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). As this product contains the Alexa Fluor® dye the sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: (i) in manufacturing; (ii) to provide a service, information, or data in return for payment (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are sold for use in research.</p>

For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008 USA or [outlicensing@thermofisher.com](mailto:outlicensing@thermofisher.com).

## Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle. Store In the Dark.
Dissociation constant ( $K_D$ )	$K_D = 3.60 \times 10^{-11}$ M



[Learn more about  \$K\_D\$](#)

Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: 30% Glycerol (glycerin, glycerine), 1% BSA, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP774Y
Isotype	IgG

## Applications

**The Abpromise guarantee** Our [Abpromise guarantee](#) covers the use of ab205828 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/100.
Flow Cyt (Intra)		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.

## Target

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,
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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.

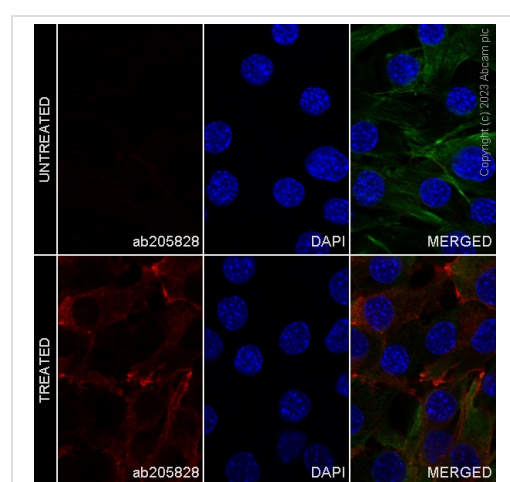
Phosphorylation at Thr-678 and Thr-693 by PRKD1 inhibits EGF-induced MAPK8/JNK1 activation. Dephosphorylation by PTPRJ prevents endocytosis and stabilizes the receptor at the plasma membrane. Autophosphorylation at Tyr-1197 is stimulated by methylation at Arg-1199 and enhances interaction with PTPN6. 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

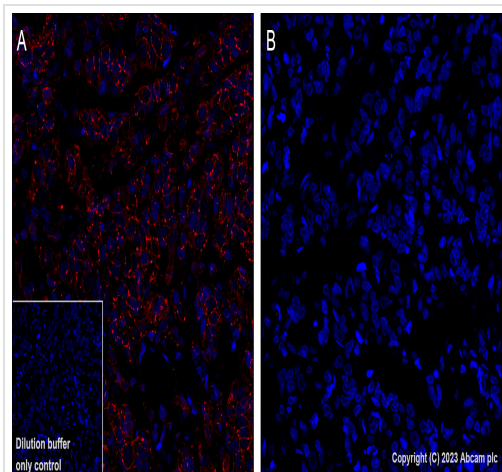


Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab205828)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized C2C12 (mouse myoblast) cells labelling EGFR (phospho Y1068) with ab205828 at 1/50 (10.0 µg/ml) dilution (Green). **ab195887** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 488) was used to counterstain tubulin at 1/200 (2.5 µg/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Confocal image showing positive staining in C2C12 cells treated with EGF (10ng/ml, 30min) and no staining on the cells without EGF treatment.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Alexa Fluor® 647 Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab205828)

Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labeling EGFR (phospho Y1068) with ab205828 at 1/100 dilution.

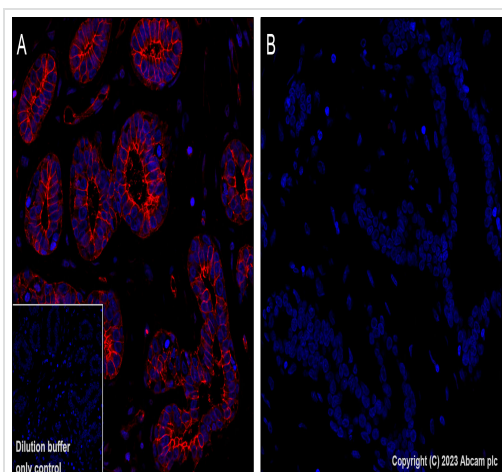
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 40 mins.

Positive staining on human breast cancer without alkaline phosphatase treatment (image A). No signal was detected when tissues were treated with alkaline phosphatase (image B).

The section was incubated with ab205828 for 60 mins at room temperature (shown in red). Nuclear DNA was labeled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

The immunostaining was performed on a Leica Biosystems BOND RX instrument.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Alexa Fluor® 647 Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab205828)

Immunohistochemical analysis of paraffin-embedded Human breast tissue labeling EGFR (phospho Y1068) with ab205828 at 1/100 dilution.

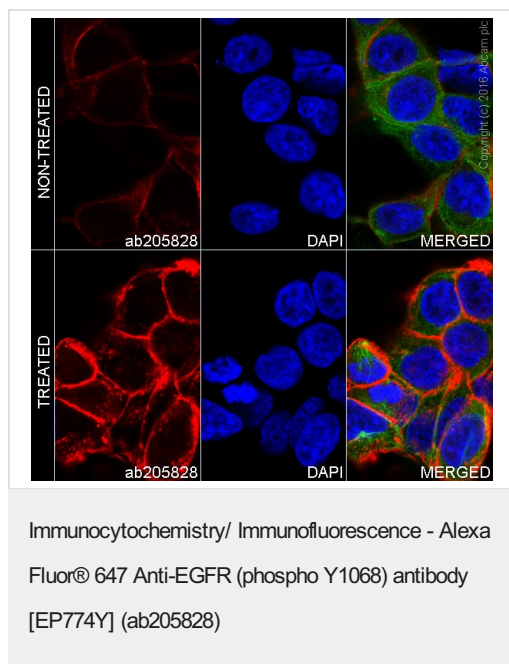
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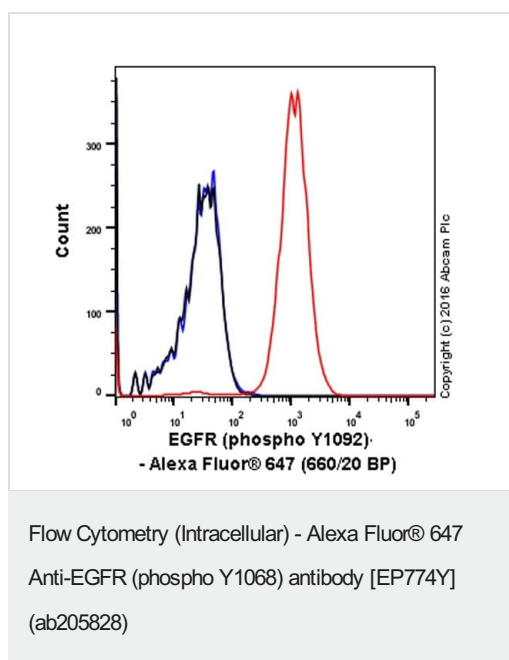
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



ab205828 staining EGFR (phospho Y1092) in A431 cells +/-EGF (100ng/ml, 5min). The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h.

The cells were then incubated overnight at +4°C with ab205828 at 1:100 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry analysis of A431 (human epidermoid carcinoma) cells labeling EGFR (phospho Y1068) with purified ab205828 at 1/500 dilution(10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 647) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Alexa Fluor® 647 Anti-EGFR (phospho Y1068)  
antibody [EP774Y] (ab205828)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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