abcam

Product datasheet

# Alexa Fluor® 647 Anti-EGFR antibody [EP38Y] ab192982

Recombinant RabMAb

3 References 4 Images

## Overview

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|---|---|
| **Product name** | Alexa Fluor® 647 Anti-EGFR antibody [EP38Y] |
| **Description** | Alexa Fluor® 647 Rabbit monoclonal [EP38Y] to EGFR |
| **Host species** | Rabbit |
| **Conjugation** | Alexa Fluor® 647. Ex: 652nm, Em: 668nm |
| **Tested applications** | **Suitable for:** ICC/IF, Flow Cyt (Intra) |
| **Species reactivity** | **Reacts with:** Human<br>**Predicted to work with:** Mouse, Rat |
| **Immunogen** | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| **Positive control** | ICC/IF: HeLa and A431 cells, Flow Cyt (intra): HeLa |
| **General notes** | Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.<br>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: 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. 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**. |

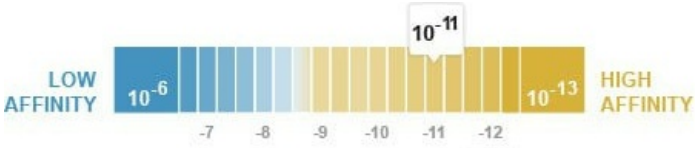
## Properties

| | |
|---|---|
| **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. Stable for 12 months at -20°C. Store In the Dark.

**Dissociation constant ($K_D$)**

$K_D = 1.90 \times 10^{-11}$ 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**

Immunogen affinity purified

**Clonality**

Monoclonal

**Clone number**

EP38Y

**Isotype**

IgG

## Applications

**The Abpromise guarantee**

Our **Abpromise guarantee** covers the use of ab192982 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** | | 1/50 - 1/1000.<br>This product gave a positive signal in HeLa cells fixed with 100% methanol (5 min) and A431 cells fixed with 4% formaldehyde (10 min) and 100% methanol (5 min). |
| **Flow Cyt (Intra)** | | Use 2µl for $10^6$ cells.<br>**ab199093** - Rabbit monoclonal IgG (Alexa Fluor® 647), is suitable for use as an isotype control with this antibody. |

## Target

**Function**

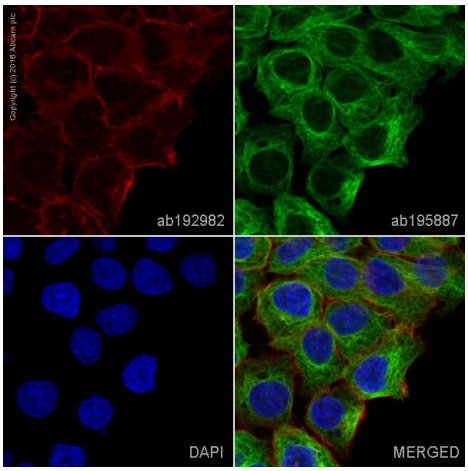
Receptor tyrosine kinase binding ligands of the EGF family and activating several signaling cascades to convert extracellular cues into appropriate cellular responses. Known ligands include EGF, TGFA/TGF-alpha, amphiregulin, epigen/EPGN, BTC/betacellulin, epiregulin/EREG and HBEGF/heparin-binding EGF. 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, 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<br>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.<br>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

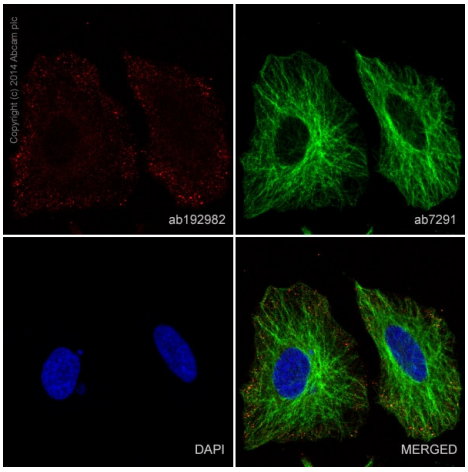



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-EGFR antibody [EP38Y] (ab192982)

ab192982 staining EGFR in A431 cells. 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 ab192982 at 1/1000 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).

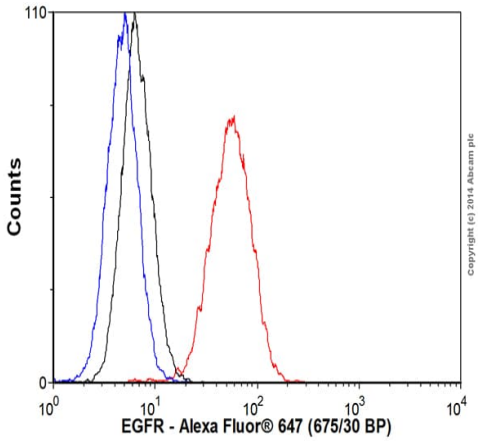
This product also gave a positive signal under the same testing conditions in A431 cells fixed with 100% methanol (5 min).




Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-EGFR antibody [EP38Y] (ab192982)

ab192982 staining EGFR in HeLa cells. The cells were fixed with 100% methanol (5 min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Triton X-100 for 1hr. The cells were then incubated with ab192982 at a working dilution of 1 in 50 (shown in red) and **ab7291** (Mouse monoclonal [DM1A] to alpha Tubulin) at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1hr with AlexaFluor® 488 Goat anti-mouse IgG (H&L - preadsorbed) (**ab150117**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

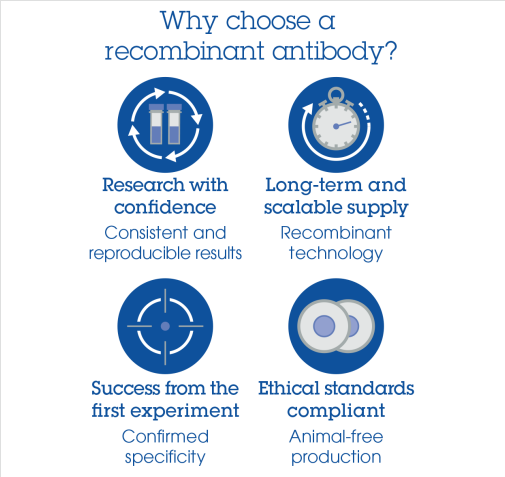
Image was taken with a Confocal microscope (Leica-microsystems, TCS SP8).




Flow Cytometry (Intracellular) - Alexa Fluor® 647 Anti-EGFR antibody [EP38Y] (ab192982)

Overlay histogram showing HeLa cells stained with ab192982 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab192982, 2µl/1x$10^6$ cells) for 30 min at 22ºC. Isotype control antibody (black line) was rabbit IgG (monoclonal) (Alexa Fluor® 647) (1µg/1x$10^6$ cells) for 30 min at 22ºC. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 25mW red solid state diode laser (635nm) and 675/30 bandpass filter.




Alexa Fluor® 647 Anti-EGFR antibody [EP38Y]

(ab192982)

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

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