

## Product datasheet

# Anti-EGFR (phospho T654) antibody [EP2255Y] ab75986

Recombinant RabMAb

## 2 Images

### Overview

<b>Product name</b>	Anti-EGFR (phospho T654) antibody [EP2255Y]
<b>Description</b>	Rabbit monoclonal [EP2255Y] to EGFR (phospho T654)
<b>Host species</b>	Rabbit
<b>Specificity</b>	Recognises EGFR phosphorylated on Threonine 654 of the mature human isoform 1 (corresponding to T678 from the precursor form P00533-1/p170)
<b>Tested applications</b>	<b>Suitable for:</b> WB, Dot blot <b>Unsuitable for:</b> Flow Cyt, IHC-P or IP
<b>Species reactivity</b>	<b>Reacts with:</b> Human
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: A431 (Human epidermoid carcinoma epithelial cell) treated with 100ng/ml EGF for 30 minutes whole cell lysate.
<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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p> <p>Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.</p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.
<b>Storage buffer</b>	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS

<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EP2255Y
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab75986 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
<b>WB</b>		1/1000 - 1/50000. Predicted molecular weight: 134 kDa.
<b>Dot blot</b>		1/1000.

**Application notes** Is unsuitable for Flow Cyt, IHC-P or IP.

## 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  
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.

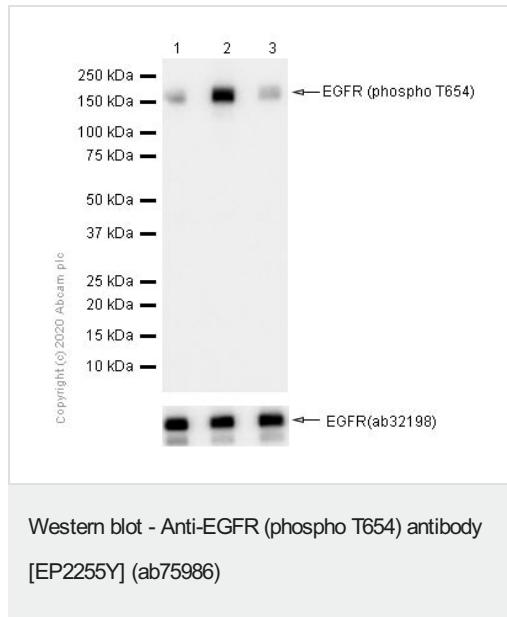
**Post-translational modifications** Phosphorylation at Ser-695 is partial and occurs only if Thr-693 is phosphorylated. Phosphorylation at Thr-678 and Thr-693 by PRKD1 inhibits EGF-induced MAPK8/JNK1 activation. Dephosphorylation by PTPRJ prevents endocytosis and stabilizes the receptor at the plasma 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



**All lanes :** Anti-EGFR (phospho T654) antibody [EP2255Y] (ab75986) at 1/1000 dilution (Purified)

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

**Lane 2 :** A431 (Human epidermoid carcinoma epithelial cell) treated with 100ng/ml EGF for 30 minutes whole cell lysate

**Lane 3 :** A431 (Human epidermoid carcinoma epithelial cell) treated with 100ng/ml EGF for 30 minutes whole cell lysate, then the membrane treated with Lambda Phosphatase for 1 hour

Lysates/proteins at 15 µg per lane.

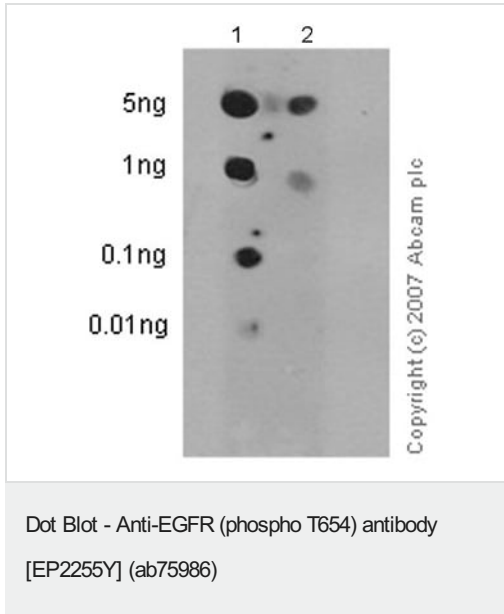
### Secondary

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

**Predicted band size:** 134 kDa

**Observed band size:** 170 kDa

Blocking buffer: 5% NFD/MTBST



Primary antibody dilution: 1/1000

Secondary antibody: goat anti-rabbit IgG, (H+L), peroxidase conjugated

Secondary antibody dilution: 1/2500

Blocking & dilution buffer: 5% NFD/MTBST

Lane 1 sample: EGFR (pT654) phospho peptide

Lane 2 sample: EGFR non-phospho peptide

Exposure time: 3 minutes

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

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