Anti-Phospho-EGFR (Y1148) Antibody

HA500580

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell
Molecular Wt:	Predicted band size: 134 kDa

Description: Epidermal growth factor receptor (EGFR) is a transmembrane protein that is activated by binding of its specific ligands, including epidermal growth factor and transforming growth factor alpha (TGF-α). ErbB2 has no known direct activating ligand, and may be in an activated state constitutively or become active upon heterodimerization with other family members such as EGFR. Upon activation by its growth factor ligands, EGFR undergoes a transition from an inactive monomeric form to an active homodimer. – although there is some evidence that preformed inactive dimers may also exist before ligand binding. In addition to forming homodimers after ligand binding, EGFR may pair with another member of the ErbB receptor family, such as ErbB2/Her2/neu, to create an activated heterodimer. There is also evidence to suggest that clusters of activated EGFRs form, although it remains unclear whether this clustering is important for activation itself or occurs subsequent to activation of individual dimers.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Tyr1148 of human EGFR.

Positive control: A431 starved overnight then treated with 20ng/mL EGF for 5 minutes cell lysate, A431 cells starved overnight then treated with 20ng/mL EGF for 5 minutes.

Subcellular location: Cell membrane, Endoplasmic reticulum membrane, Golgi apparatus membrane, Nucleus membrane, Endosome, Endosome membrane, Nucleus; Secreted.

Database links: SwissProt: P00533 Human

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:10,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

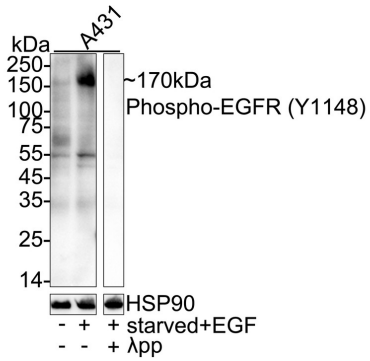

Fig1: Western blot analysis of Phospho-EGFR (Y1148) on different lysates with Rabbit anti-Phospho-EGFR (Y1148) antibody (HA500580) at 1/5,000 dilution.

Lane 1: A431 cell lysate
Lane 2: A431 starved overnight then treated with 20ng/mL EGF for 5 minutes cell lysate
Lane 3: A431 starved overnight then treated with 20ng/mL EGF for 5 minutes cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 1 minute 23 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA500580) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

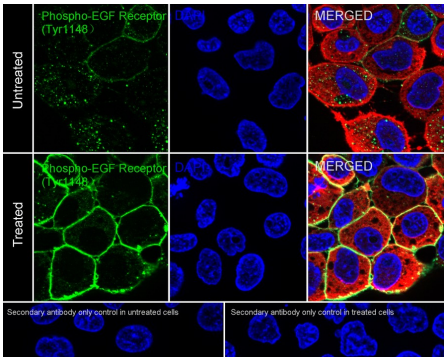

Fig2: Immunocytochemistry analysis of A431 cells untreated / starved overnight then treated with 20ng/mL EGF for 5 minutes labeling Phospho-EGFR (Y1148) with Rabbit anti-Phospho-EGFR (Y1148) antibody (HA500580) at 1/10,000 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-EGFR (Y1148) antibody (HA500580) at 1/10,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Background References

1. Levantini E et al. EGFR signaling pathway as therapeutic target in human cancers. Semin Cancer Biol. 2022 Oct
2. Schramm F et al. EGFR Signaling in Lung Fibrosis. Cells. 2022 Mar