Anti-Phospho-EGFR (Y1045) Antibody [PSH03-28] HA721970

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 134 kDa
Clone number:	PSH03-28

Description: The epidermal growth factor receptor (EGFR; ErbB-1; HER1 in humans) is a transmembrane protein that is a receptor for members of the epidermal growth factor family (EGF family) of extracellular protein ligands. The epidermal growth factor receptor is a member of the ErbB family of receptors, a subfamily of four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4). In many cancer types, mutations affecting EGFR expression or activity could result in cancer. Deficient signaling of the EGFR and other receptor tyrosine kinases in humans is associated with diseases such as Alzheimer's, while over-expression is associated with the development of a wide variety of tumors. Interruption of EGFR signalling, either by blocking EGFR binding sites on the extracellular domain of the receptor or by inhibiting intracellular tyrosine kinase activity, can prevent the growth of EGFR-expressing tumours and improve the patient's condition.

Immunogen: Synthetic phosphopeptide corresponding to residues surrounding Tyr1045 of EGFR.

Positive control: A431 treated with 100ng/mL EGF for 30 minutes cell lysate, A431 treated with 100ng/mL EGF for 60 minutes.

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

Database links: SwissProt: P00533 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
FC	1:1,000

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

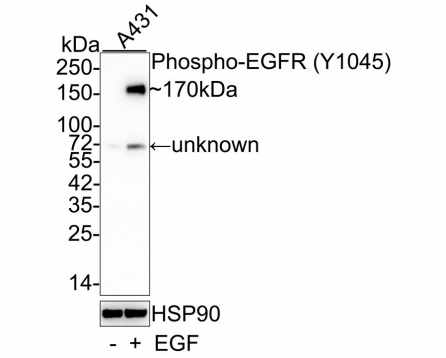

Fig1: Western blot analysis of Phospho-EGFR (Y1045) on different lysates with Rabbit anti-Phospho-EGFR (Y1045) antibody (HA721970) at 1/1,000 dilution.

Lane 1: A431 cell lysate
Lane 2: A431 treated with 100ng/mL EGF for 30 minutes cell lysate

Lysates/proteins at 30 µg/Lane.

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

Exposure time: 3 minutes 10 seconds;

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 (HA721970) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

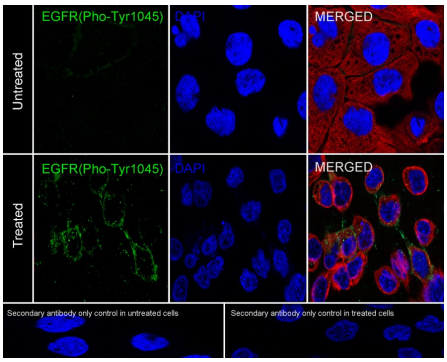

Fig2: Immunocytochemistry analysis of A431 treated with or without 100ng/mL EGF for 60 minutes cells labeling Phospho-EGFR (Y1045) with Rabbit anti-Phospho-EGFR (Y1045) antibody (HA721970) at 1/100 dilution.

Cells were fixed in 100% precooled methanol for 5 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 (Y1045) antibody (HA721970) at 1/100 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 (M1305-2, 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.

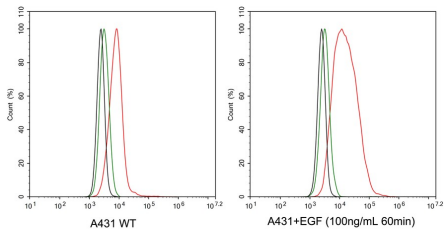

Fig3: Flow cytometric analysis of A431 treated with or without 100ng/mL EGF for 60 minutes cells labeling Phospho-EGFR (Y1045).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721970, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Nakamura T et al. LRIG1 inhibits STAT3-dependent inflammation to maintain corneal homeostasis. J Clin Invest 124:385-97 (2014).
2. Furcht CM et al. Multivariate signaling regulation by SHP2 differentially controls proliferation and therapeutic response in glioma cells. J Cell Sci 127:3555-67 (2014).