Anti-Phospho-EGFR (T669) Antibody [PSH10-03] - BSA and Azide free

HA751333

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IP
Molecular Wt:	Predicted band size: 134 kDa
Clone number:	PSH10-03

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 phospho-peptide corresponding to residues surrounding Thr669 of Human EGFR.

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

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

Database links: SwissProt: P00533 Human

Recommended Dilutions:

WB	1:5,000
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

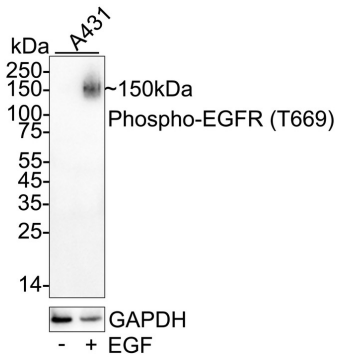

Fig1: Western blot analysis of Phospho-EGFR (T669) on different lysates with Rabbit anti-Phospho-EGFR (T669) antibody (HA751333) at 1/5,000 dilution.

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

Lysates/proteins at 20 µg/Lane.

Predicted band size: 134 kDa
Observed band size: 150 kDa

Exposure time: 3 minutes; 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 (HA751333) at 1/5,000 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.

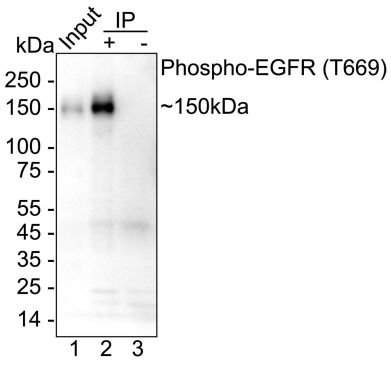

Fig2: Phospho-EGFR (T669) was immunoprecipitated from 0.2 mg A431 starved overnight then treated with 100ng/mL EGF for 15 minutes cell lysate with HA751333 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA751333 at 1/2,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: A431 starved overnight then treated with 100ng/mL EGF for 15 minutes cell lysate (input)
Lane 2: HA751333 IP in A431 starved overnight then treated with 100ng/mL EGF for 15 minutes cell lysate
Lane 3: Rabbit IgG instead of HA751333 in A431 starved overnight then treated with 100ng/mL EGF for 15 minutes cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)
Exposure time: 59 seconds; ECL: K1801

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).