Anti-Phospho-EGFR (Y1068) Antibody [SD2055]

ET1612-30

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IP, FC
Molecular Wt:	Predicted band size: 134 kDa
Clone number:	SD2055

Description: Epidermal growth factor mediates its effects on cell growth through its inter-action with a cell surface glycoprotein designated the EGF receptor. Binding of EGF or TGF alpha to the EGF receptor activates tyrosine-specific protein kinase activity intrinsic to the EGF receptor. The carboxy terminal tyrosine residues on EGFR, Tyr 1068 and Tyr 1173, are the major sites of autophosphorylation, which occurs as a result of EGF binding. Once activated, EGFR mediates the binding of the phosphotyrosine binding (PTB) domain of GRB2 through direct interactions with Tyr 1068 and Tyr 1086 and through indirect interactions with Tyr 1173 in the Ras signaling pathway. Tyr 1173 of EGFR also functions as a kinase substrate. Phosphorylation of Tyr 992, Tyr 1068 and Tyr 1086 is required for conformational change in the C-terminal tail of the EGF receptor.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Tyr1068 of human EGFR.

Positive control: HeLa cell lysate, HeLa treated with 100ng/mL EGF for 30 minutes cell lysate, A431, Hela, MCF-7.

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

Database links: SwissProt: P00533 Human

Recommended Dilutions:

WB	1:500-1:5,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
FC	1:50-1:100
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

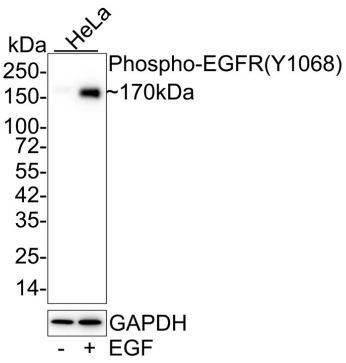

Fig1: Western blot analysis of Phospho-EGFR (Y1068) on different lysates with Rabbit anti-Phospho-EGFR (Y1068) antibody (ET1612-30) at 1/5,000 dilution.

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

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 24 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 (ET1612-30) 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.

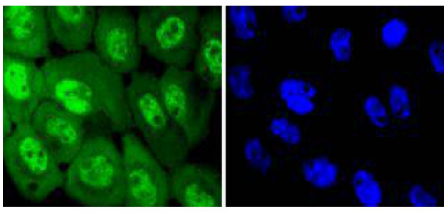

Fig2: ICC staining of Phospho-EGFR (Y1068) in A431 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1612-30, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

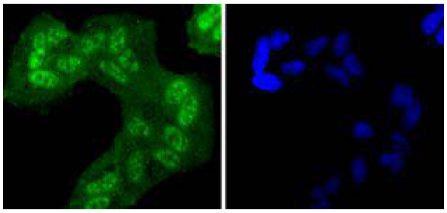

Fig3: ICC staining of Phospho-EGFR (Y1068) in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1612-30, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

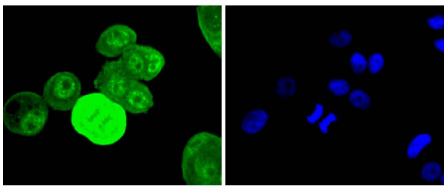

Fig4: ICC staining of Phospho-EGFR (Y1068) in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1612-30, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

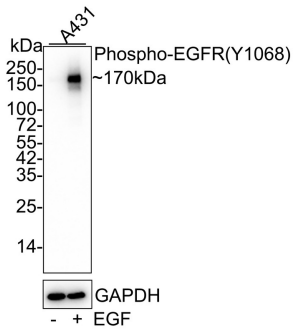

Fig5: Western blot analysis of Phospho-EGFR (Y1068) on different lysates with Rabbit anti-Phospho-EGFR (Y1068) antibody (ET1612-30) at 1/5,000 dilution.

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: 170 kDa

Exposure time: 28 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 (ET1612-30) 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.

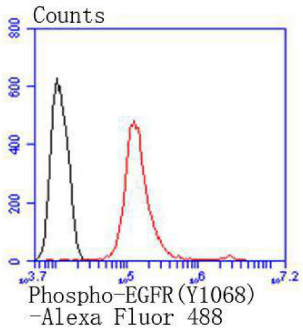

Fig6: Flow cytometric analysis of Phospho-EGFR (Y1068) was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1612-30, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Zhang W et al. A Selected Lactobacillus rhamnosus Strain Promotes EGFR-Independent Akt Activation in an Enterotoxigenic Escherichia coli K88-Infected IPEC-J2 Cell Model. PLoS One 10:e0125717 (2015).
2. Pan T et al. Cytohesins/ARNO: the function in colorectal cancer cells. PLoS One 9:e90997 (2014).