Anti-Phospho-EGFR (Y1173) Antibody [SC57-04] ET1610-4

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, IHC-P, IP
Molecular Wt:	Predicted band size: 134 kDa
Clone number:	SC57-04

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 Tyr1173 of Human EGFR.

Positive control: A431 treated with 100ng/mL EGF for 30 minutes whole cell lysate, A431, B16F1, human placenta tissue, mouse skin tissue.

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

Database links: SwissProt: P00533 Human | Q01279 Mouse

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200
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: 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

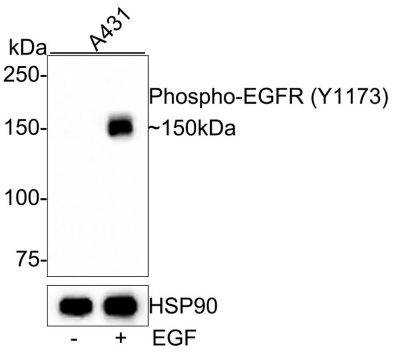

Fig1: Western blot analysis of Phospho-EGFR (Y1173) on different lysates with Rabbit anti-Phospho-EGFR (Y1173) antibody (ET1610-4) at 1/500 dilution.

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

Lysates/proteins at 10 µg/Lane.

Predicted band size: 135 kDa
Observed band size: 150 kDa

Exposure time: 1 minute;
6% 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 (ET1610-4) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/200,000 dilution was used for 1 hour at room temperature.

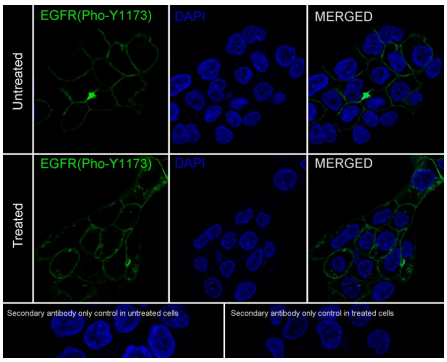

Fig2: Immunocytochemistry analysis of A431 cells treated with or without 100ng/mL EGF for 5 minutes labeling Phospho-EGFR (Y1173) with Rabbit anti-Phospho-EGFR (Y1173) antibody (ET1610-4) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at 37 ℃, permeabilized with 0.1% Triton X-100 in PBS permeabilization for 5 minutes, and then blocked with 2% negative goat serum for 60 minutes at room temperature. Cells were then incubated with Rabbit anti-Phospho-EGFR (Y1173) antibody (ET1610-4) at 1/50 dilution in 1% BSA 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.

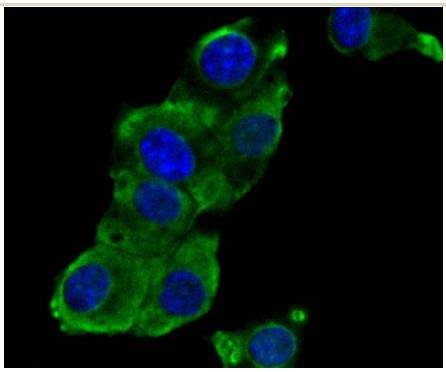

Fig3: ICC staining of Phospho-EGFR (Y1173) in untreated B16F1 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1610-4, 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).

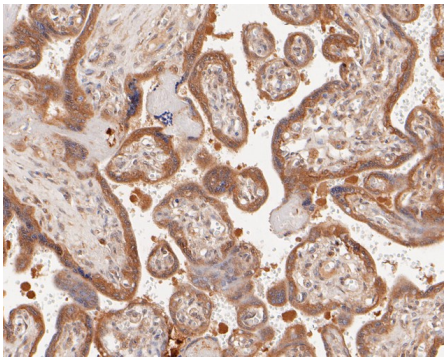

Fig4: Immunohistochemical analysis of paraffin-embedded human placenta tissue using anti-Phospho-EGFR (Y1173) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-4, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

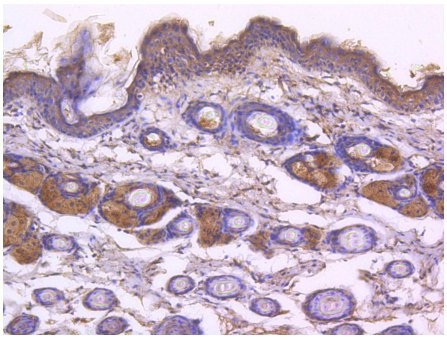

Fig5: Immunohistochemical analysis of paraffin-embedded mouse skin tissue using anti-Phospho-EGFR (Y1173) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-4, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Chang YL et al. Regulation of estrogen receptor a function in oral squamous cell carcinoma cells by FAK signaling. Endocr Relat Cancer 21:555-65 (2014).
2. Gerdes CA et al. GA201 (RG7160): a novel, humanized, glycoengineered anti-EGFR antibody with enhanced ADCC and superior in vivo efficacy compared with cetuximab. Clin Cancer Res 19:1126-38 (2013).