

Anti-EGFR Antibody [SZ40-19]

ET1603-37



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Monkey, Rat
Applications:	WB, IF-Cell, IP, FC, mIHC
Molecular Wt:	Predicted 134 kDa, observed 150 kDa.
Clone number:	SZ40-19

Description: The EGF receptor family comprises several related receptor tyrosine kinases that are frequently overexpressed in a variety of carcinomas. Members of this receptor family include EGFR (HER1), Neu (ErbB-2, HER2), ErbB-3 (HER3) and ErbB-4 (HER4), which form either homodimers or heterodimers upon ligand binding. Exons in the EGFR gene product are frequently either deleted or duplicated to produce deletion mutants (DM) or tandem duplication mutants (TDM), respectively, which are detected at various molecular weights. EGFR binds several ligands, including epidermal growth factor (EGF), transforming growth factor α (TGF α), Amphiregulin and heparin binding-EGF (HB-EGF). Ligand binding promotes the internalization of EGFR via Clathrin-coated pits and its subsequent degradation in response to its intrinsic tyrosine kinase. EGFR is involved in organ morphogenesis and maintenance and repair of tissues, but upregulation of EGFR is associated with tumor progression. The oncogenic effects of EGFR include initiation of DNA synthesis, enhanced cell growth, invasion and metastasis. Abrogation of EGFR results in cell cycle arrest, apoptosis or dedifferentiation of cancer cells, suggesting that EGFR may be an effective therapeutic target.

Immunogen: Synthetic peptide within human EGFR aa 1070-1110.

Positive control: HeLa cell lysate, A431 cell lysate, MDA-MB-468 cell lysate, A431, Hela, HepG2, human lung cancer tissue, human kidney tissue.

Subcellular location: Secreted and Cell membrane.

Database links: SwissProt: P00533 Human | Q01279 Mouse
Unigene: 37227 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:500
IP	1:10-1:50
FC	1:1,000
mIHC	1:1,000

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

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

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

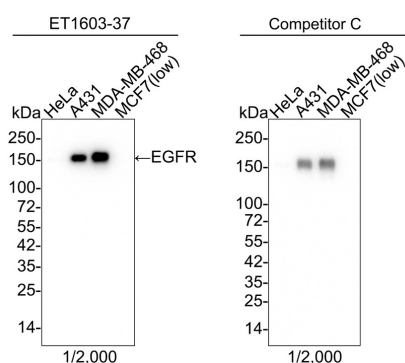


Fig1: Western blot analysis of EGFR on different lysates with Rabbit anti-EGFR antibody (ET1603-37) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: HeLa cell lysate

Lane 2: A431 cell lysate

Lane 3: MDA-MB-468 cell lysate

Lane 4: MCF7 cell lysate (low expression)

Lysates/proteins at 15 µg/Lane.

Predicted band size: 134 kDa

Observed band size: 150 kDa

Exposure time: 21 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 (ET1603-37) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution were used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Western blot analysis of EGFR on different lysates with Rabbit anti-EGFR antibody (ET1603-37) at 1/2,000 dilution.

Lane 1: A431-si NT cell lysate

Lane 2: A431-si EGFR cell lysate

Lysates/proteins at 10 µg/Lane.

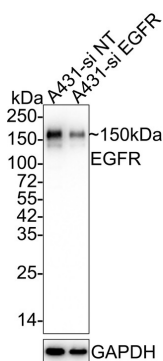
Predicted band size: 134 kDa

Observed band size: 150 kDa

Exposure time: 2 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 (ET1603-37) at 1/2,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



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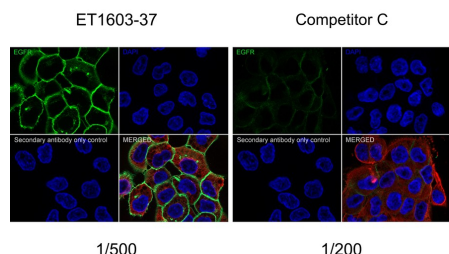
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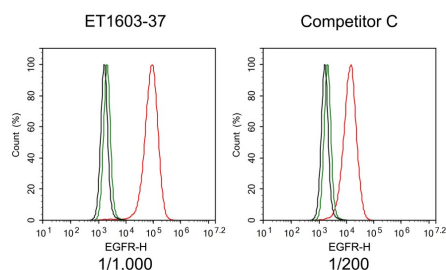
Fig3: Immunocytochemistry analysis of A431 cells labeling EGFR with Rabbit anti-EGFR antibody (ET1603-37) at 1/500 dilution and competitor's antibody at 1/200 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS 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-EGFR antibody (ET1603-37) at 1/500 dilution and competitor's antibody at 1/200 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig4: Flow cytometric analysis of A431 cells labeling EGFR.



Cells were fixed and permeabilized. Then stained with the primary antibody (ET1603-37, red) at 1/1,000 dilution and competitor's antibody (red) at 1/200 dilution, compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

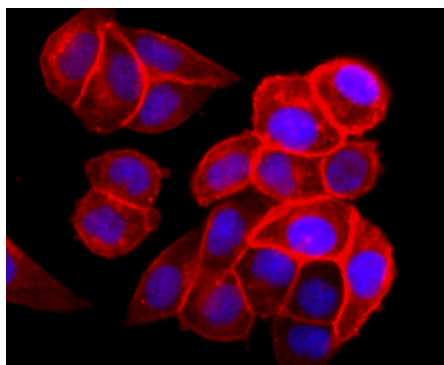


Fig5: ICC staining of EGFR in Hela cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1603-37, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

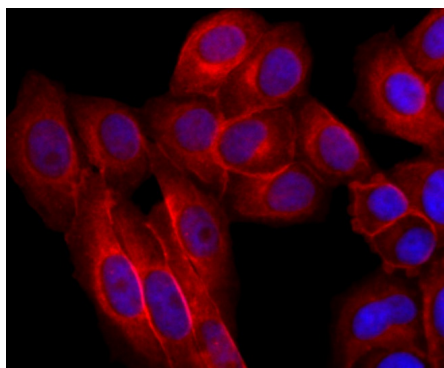


Fig6: ICC staining of EGFR in HepG2 cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1603-37, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

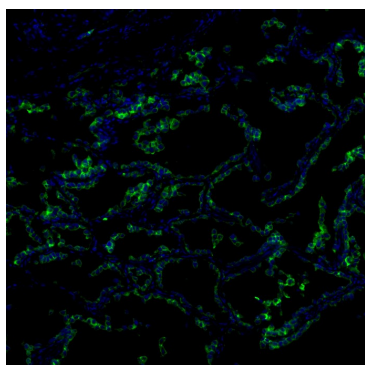


Fig7: mIHC analysis of human lung cancer tissue (Formalin/PFA-fixed paraffin-embedded sections) with Rabbit anti-EGFR antibody (ET1603-37) at 1/1,000 dilution. The immunostaining was performed with the IRISKit® HyperView mTSA Kit (MH900206). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

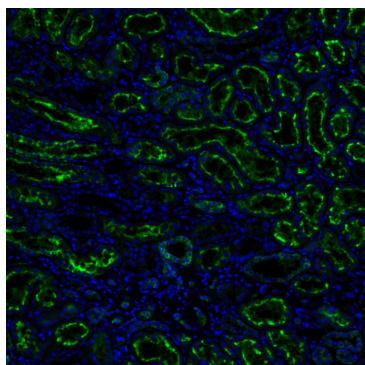


Fig8: mIHC analysis of human kidney cortex tissue (Formalin/PFA-fixed paraffin-embedded sections) with Rabbit anti-EGFR antibody (ET1603-37) at 1/1,000 dilution. The immunostaining was performed with the IRISKit® HyperView mTSA Kit (MH900206). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

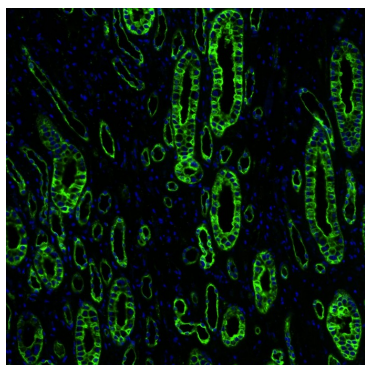


Fig9: mIHC analysis of human kidney medulla tissue (Formalin/PFA-fixed paraffin-embedded sections) with Rabbit anti-EGFR antibody (ET1603-37) at 1/1,000 dilution. The immunostaining was performed with the IRISKit® HyperView mTSA Kit (MH900206). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

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

Background References

1. Chen CC et al. The matricellular protein CCN1 suppresses hepatocarcinogenesis by inhibiting compensatory proliferation. *Oncogene* 35:1314-23 (2016).
2. Desai TJ et al. Alveolar progenitor and stem cells in lung development, renewal and cancer. *Nature* 507:190-4 (2014).

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