Anti-EGFR Antibody [SP00-86]

ET1604-44

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	134 kDa
Clone number:	SP00-86

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 (TGFα), 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, human tonsil tissue, human breast carcinoma tissue, mouse brain tissue, mouse skin tissue.

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

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

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
IHC-P	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

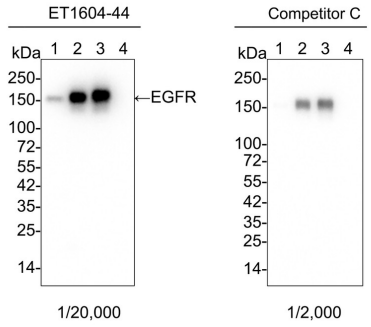

Fig1: Western blot analysis of EGFR on different lysates with Rabbit anti-EGFR antibody (ET1604-44) at 1/20,000 dilution and competitor's antibody at 1/2,000 dilution.

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: 175 kDa

Exposure time: 21 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 (ET1604-44) at 1/20,000 dilution and competitor's antibody at 1/2,000 dilution were 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.

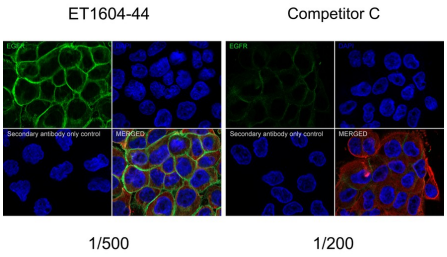

Fig2: Immunocytochemistry analysis of A431 cells labeling EGFR with Rabbit anti-EGFR antibody (ET1604-44) at 1/500 dilution and competitor's antibody at 1/200 dilution.

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 (ET1604-44) at 1/500 dilution and competitor's antibody at 1/200 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.

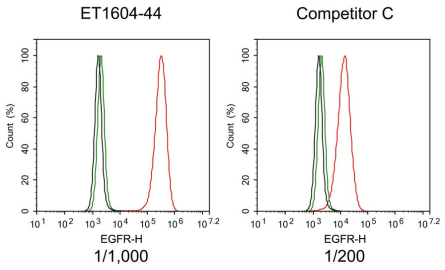

Fig3: Flow cytometric analysis of A431 cells labeling EGFR.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1604-44, 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℃ 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).

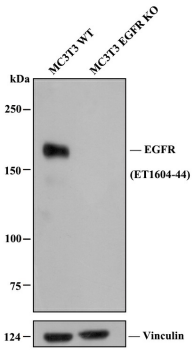

Fig4: All lanes: Western blot analysis of EGFR with anti-EGFR antibody [SP00-86] (ET1604-44) at 1:500 dilution.

Lane 1: Wild-type MC3T3 whole cell lysate.
Lane 2: EGFR knockout MC3T3 whole cell lysate.

Predicted band size: 134 kDa
Observed band size: 180 kDa

ET1604-44 was shown to specifically react with EGFR in wild-type MC3T3 cells. No band was observed when EGFR knockout samples were tested. Wild-type and EGFR knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary Anti-EGFR antibody (ET1604-44, 1/500) and Anti-Vinculin antibody (ET1705-94, 1/5,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG H&L (HRP) Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

Cell lysate was provided by Ubigene Biosciences (Ubigene Biosciences Co., Ltd., Guangzhou, China).

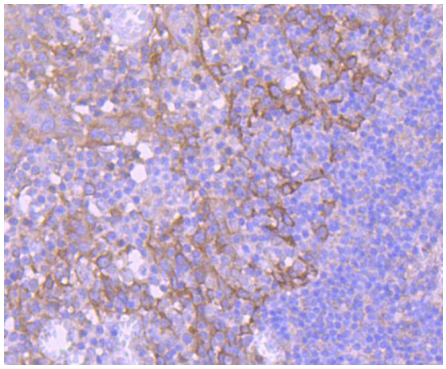

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-EGFR antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1604-44, 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.

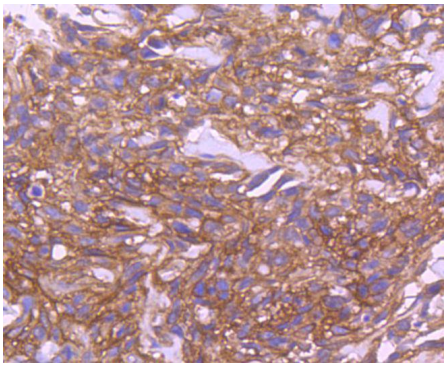

Fig6: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-EGFR antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1604-44, 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.

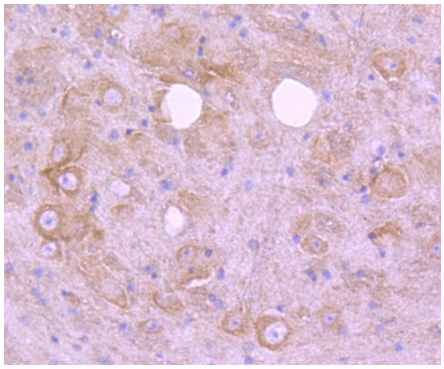

Fig7: Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-EGFR antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1604-44, 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.

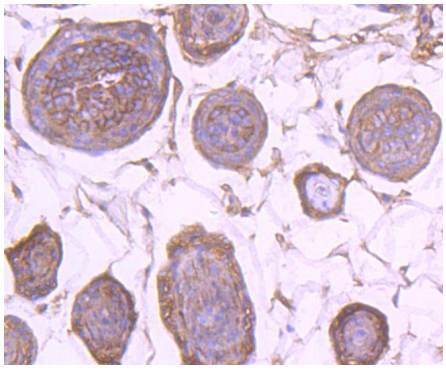

Fig8: Immunohistochemical analysis of paraffin-embedded mouse skin tissue using anti-EGFR antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1604-44, 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.

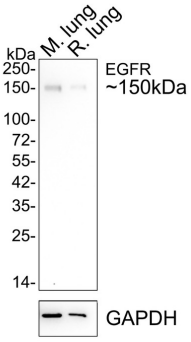

Fig9: Western blot analysis of EGFR on different lysates with Rabbit anti-EGFR antibody (ET1604-44) at 1/1,000 dilution.

Lane 1: Mouse lung tissue lysate
Lane 2: Rat lung tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 134 kDa
Observed band size: 175 kDa

Exposure time: 20 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 (ET1604-44) 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.

Background References

1. Chen CC et al. The matricellular protein CCN1 suppresses hepatocarcinogenesis by inhibiting compensatory proliferation. Oncogene 35:1314-23 (2016).
2. Fu Y et al. Ductal activation of oncogenic KRAS alone induces sarcomatoid phenotype. Sci Rep 5:13347 (2015).