Anti-EGFR Antibody [7-F2-F8]

EM1901-67

Product Type:	Mouse monoclonal IgG2b, primary antibodies
Species reactivity:	Human, Monkey
Applications:	WB, IHC-P, FC
Molecular Wt:	Predicted band size: 134 kDa
Clone number:	7-F2-F8

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. 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: Recombinant protein within human EGFR aa 900-1150.

Positive control: HeLa cell lysate, A431 cell lysate, MDA-MB-468 cell lysate, Wild-type MC3T3 whole cell lysate, human breast carcinoma tissue, human placenta tissue, A431.

Subcellular location: Golgi apparatus membrane, nucleus membrane, nucleus, cell membrane, endosome, endosome membrane, endoplasmic reticulum membrane, cytoplasm.

Database links: SwissProt: P00533 Human

Recommended Dilutions:

WB	1:2,000
IHC-P	1:50-1:200
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% 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

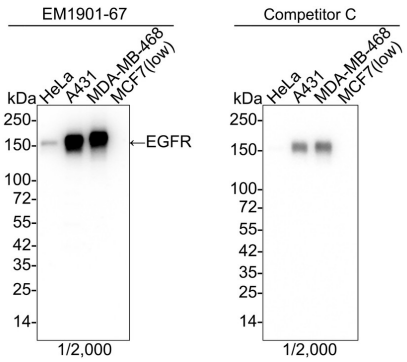

Fig1: Western blot analysis of EGFR on different lysates with Mouse anti-EGFR antibody (EM1901-67) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

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: Lane 1-4 (left): 5 seconds; Lane 1-4 (right): 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 (EM1901-67) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

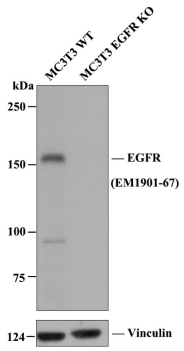

Fig2: All lanes: Western blot analysis of EGFR with anti-EGFR antibody [7-F2-F8] (EM1901-67) at 1/500 dilution.

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

EM1901-67 was shown to specifically react with EGFR in Wild-type MC3T3 cells. No band was observed when EGFR knockout sample was 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 (EM1901-67, 1/500) and Anti-Vinculin antibody (ET1705-94, 1/5,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG HRP Secondary Antibody (HA1006) at 1:20,000 dilution was used for 1 hour at room temperature.

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

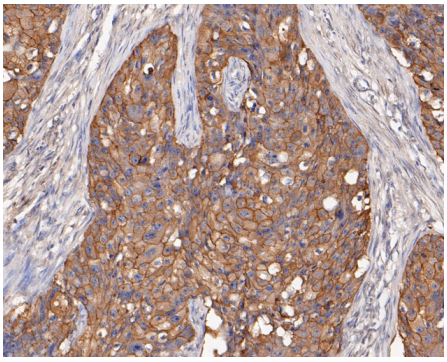

Fig3: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-EGFR antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-67, 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.

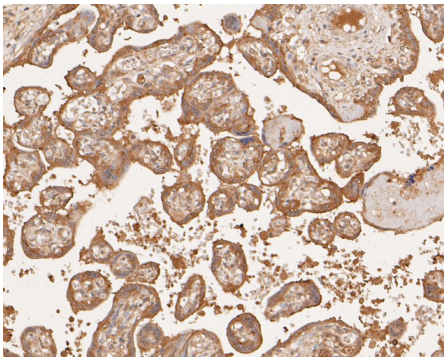

Fig4: Immunohistochemical analysis of paraffin-embedded human placenta tissue using anti-EGFR antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (EM1901-67, 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.

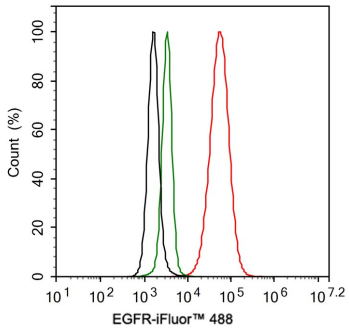

Fig5: Flow cytometric analysis of A431 cells labeling EGFR.

Cells were fixed and permeabilized. Then stained with the primary antibody (EM1901-67, 1μg/mL) (red) compared with Mouse IgG1 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-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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

Background References

1. Liu X. et. al. Epidermal growth factor receptor (EGFR): A rising star in the era of precision medicine of lung cancer. Oncotarget. 2017 Jul 25;8(30):50209-50220.
2. Singh D. et. al. Review on EGFR Inhibitors: Critical Updates. Mini Rev Med Chem. 2016;16(14):1134-66.