Anti-FGFR3 Antibody [JM81-10] - BSA and Azide free

HA750379

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 88 kDa
Clone number:	JM81-10

Description: Acidic and basic fibroblast growth factors (FGFs) are members of a family of multifunctional polypeptide growth factors that stimulate proliferation of cells of mesenchymal, epithelial and neuroectodermal origin. Like other growth factors, FGFs act by binding and activating specific cell surface receptors. These include the Flg receptor or FGFR-1, the Bek receptor or FGFR-2, FGFR-3, FGFR-4, FGFR-5 and FGFR-6. These receptors usually contain an extracellular ligand-binding region containing three immunoglobulin-like domains, a transmembrane domain and a cytoplasmic tyrosine kinase domain. The gene encoding human FGFR-3 maps to chromosome 4p16 and is alternatively spliced to produce three isoforms that are expressed in brain, kidney and testis. Defects in FGFR-3 are associated with several diseases, including Crouzon syndrome, achondroplasia, thanatophoric dysplasia, craniosynostosis adelaide type and hypochondroplasia. Mutations in FGFR-3 are also a cause of some bladder and cervical cancers.

Immunogen: Recombinant protein within Human FGFR3 aa 719-806 / 806.

Positive control: HepG2 cell lysate, HEK-293 cell lysate, LNCaP cell lysate, A549 cell lysate, Hela, MCF-7, SH-SY5Y, human breast carcinoma tissue, human kidney tissue, HepG2.

Subcellular location: Endoplasmic reticulum, Cell membrane, Cytoplasmic vesicle, Secreted.

Database links: SwissProt: P22607 Human

Recommended Dilutions:

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

Storage Buffer: 1*PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

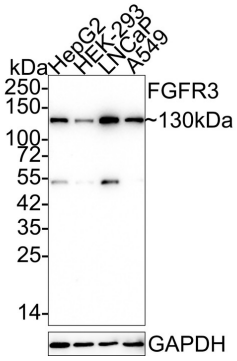

Fig1: Western blot analysis of FGFR3 on different lysates with Rabbit anti-FGFR3 antibody (HA750379) at 1/5,000 dilution.

Lane 1: HepG2 cell lysate (15 µg/Lane)
Lane 2: HEK-293 cell lysate (15 µg/Lane)
Lane 3: LNCaP cell lysate (15 µg/Lane)
Lane 4: A549 cell lysate (15 µg/Lane)

Predicted band size: 88 kDa
Observed band size: 130 kDa

Exposure time: 3 minutes;

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 (HA750379) 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.

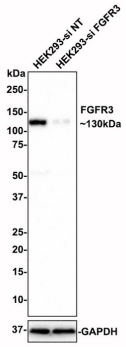

Fig2: Western blot analysis of FGFR3 on different lysates with Rabbit anti-FGFR3 antibody (HA750379) at 1/1,000 dilution.

Lane 1: HEK293-si NT cell lysate
Lane 2: HEK293-si FGFR3 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 88 kDa
Observed band size: 130 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

ET1703-36 was shown to specifically react with FGFR3 in HEK293-si NT cells. Weakened band was observed when HEK293-si FGFR3 sample was tested. HEK293-si NT and HEK293-si FGFR3 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 antibody (ET1703-36, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

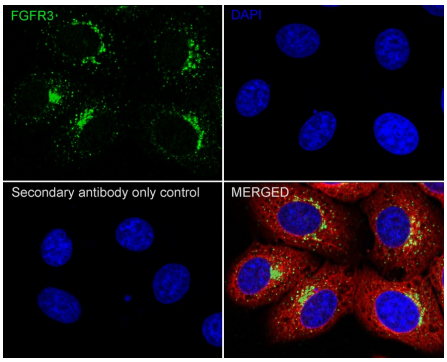

Fig3: Immunocytochemistry analysis of HepG2 cells labeling FGFR3 with Rabbit anti-FGFR3 antibody (HA750379) at 1/100 dilution.

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-FGFR3 antibody (HA750379) at 1/100 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.

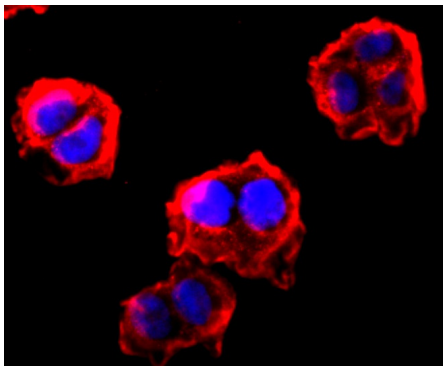

Fig4: ICC staining of FGFR3 in Hela cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750379, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

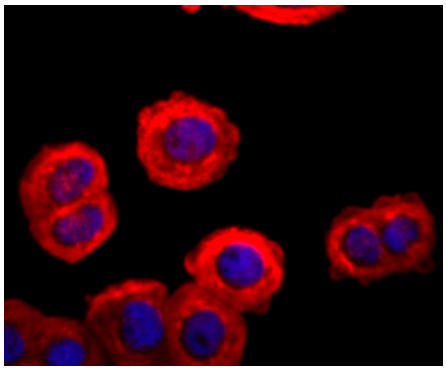

Fig5: ICC staining of FGFR3 in MCF-7 cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750379, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

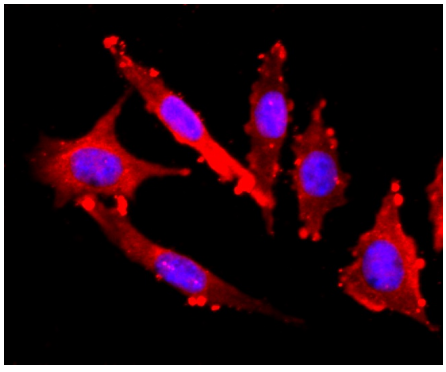

Fig6: ICC staining of FGFR3 in SH-SY5Y cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750379, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

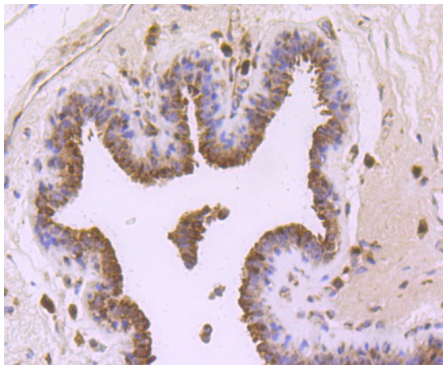

Fig7: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-FGFR3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750379, 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.

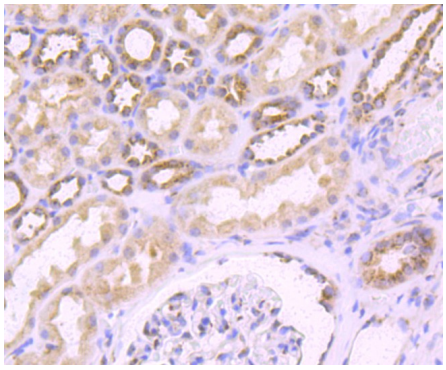

Fig8: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-FGFR3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750379, 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.

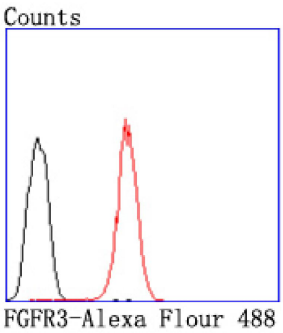

Fig9: Flow cytometric analysis of FGFR3 was done on HepG2 cells. The cells were fixed, permeabilized and stained with the primary antibody (HA750379, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

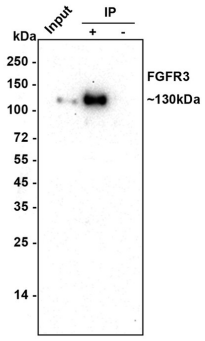

Fig10: FGFR3 was immunoprecipitated in 0.2mg HepG2 cell lysate with HA750379 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA750379 at 1/5,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HepG2 cell lysate (input)
Lane 2: HA750379 IP in HepG2 cell lysate
Lane 3: Rabbit IgG instead of HA750379 in HepG2 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 4 mintues

Background References

1. Wolk K et al. Deficient cutaneous antibacterial competence in cutaneous T-cell lymphomas: role of Th2-mediated biased Th17 function. Clin Cancer Res 20:5507-16 (2014).
2. Chung TW et al. Lipocalin-2 elicited by advanced glycation end-products promotes the migration of vascular smooth muscle cells. Biochim Biophys Acta 1833:3386-95 (2013).