Anti-VEGF Receptor 1 Antibody [SY09-09] ET1605-11

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:	Predicted band size: 151 kDa
Clone number:	SY09-09

Description: Three cell membrane receptor tyrosine kinases, Flt (also designated VEGF-R1), Flk-1 (also designated VEGF-R2) and Flt-4, putatively involved in the growth of endothelial cells, are characterized by the presence of seven immunoglobulin-like sequences in their extracellular domain. These receptors exhibit high degrees of sequence relatedness to each other as well as lesser degrees of relatedness to the class III receptors including CSF-1/Fms, PDGR, SLFR/Kit and Flt-3/Flk-2. Two members of this receptor class, Flt-1 and Flk-1, have been shown to represent high affinity receptors for vascular endothelial growth factors (VEGFs). On the basis of structural similarity to Flt and Flk-1, it has been speculated that Flt-4 might represent a third receptor for either VEGF or a VEGF-related ligand.

Immunogen: Synthetic peptide within Human VEGF Receptor 1 aa 1-50 / 1,338.

Positive control: MCF7 cell lysate, human brain tissue lysate, mouse brain tissue lysate, rat brain tissue lysate, N2A, RH-35, SHG-44, mouse placenta tissue, mouse brain tissue, A431.

Subcellular location: Cell membrane, Endosome, Secreted, Cytoplasm.

Database links: SwissProt: P17948 Human | P35969 Mouse | P53767 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:50-1:100
IF-Tissue	1:50-1:100
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

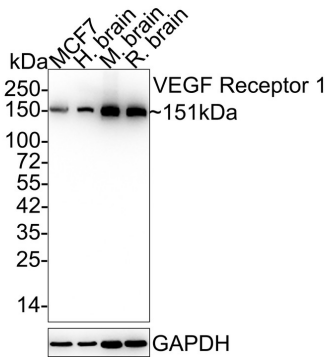

Fig1: Western blot analysis of VEGF Receptor 1 on different lysates with Rabbit anti-VEGF Receptor 1 antibody (ET1605-11) at 1/5,000 dilution.

Lane 1: MCF7 cell lysate (20 µg/Lane)
Lane 2: Human brain tissue lysate (20 µg/Lane)
Lane 3: Mouse brain tissue lysate (20 µg/Lane)
Lane 4: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 151 kDa
Observed band size: 151 kDa

Exposure time: 24 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 (ET1605-11) 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.

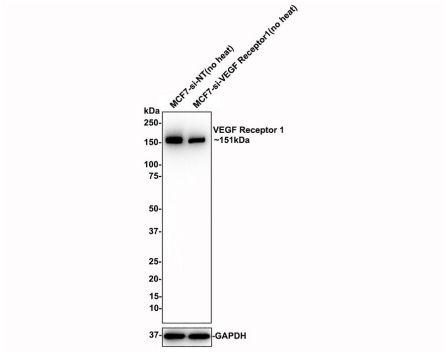

Fig2: Western blot analysis of VEGF Receptor 1 on different lysates with Rabbit anti-VEGF Receptor 1 antibody (ET1605-11) at 1/1,000 dilution.

Lane 1: MCF7-si NT cell lysate (10 µg/Lane)
Lane 2: MCF7-si VEGF Receptor 1 cell lysate (10 µg/Lane)

Predicted band size: 151 kDa
Observed band size: 151 kDa

Exposure time: 25 seconds;

4-20% SDS-PAGE gel.

ET1605-11 was shown to specifically react with VEGF Receptor 1 in Hela-si NT cells. Weakened band was observed when Hela-si VEGF Receptor 1 sample was tested. Hela-si NT and Hela-si VEGF Receptor 1 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 (ET1605-11, 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 1:100,000 dilution was used for 1 hour at room temperature.

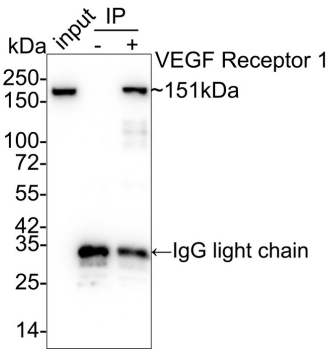

Fig3: VEGF Receptor 1 was immunoprecipitated in 0.2mg MCF7 cell lysate with ET1605-11 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1605-11 at 1/5,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: MCF7 cell lysate (input)
Lane 2: Rabbit IgG instead of ET1605-11 in MCF7 cell lysate
Lane 3: ET1605-11 IP in MCF7 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 24 seconds

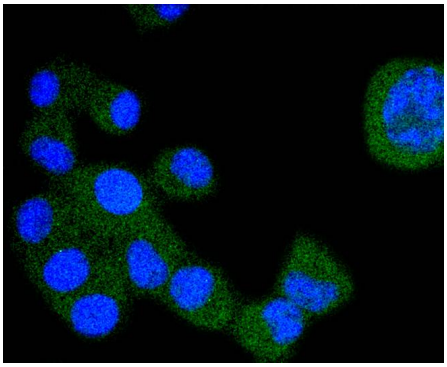

Fig4: ICC staining of VEGF Receptor 1 in N2A cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1605-11, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

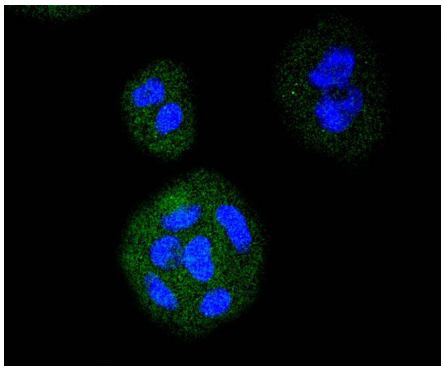

Fig5: ICC staining of VEGF Receptor 1 in RH-35 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1605-11, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

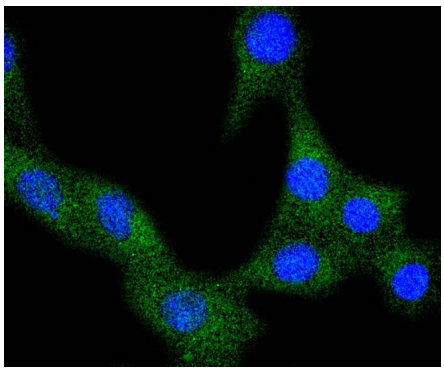

Fig6: ICC staining of VEGF Receptor 1 in SHG-44 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1605-11, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

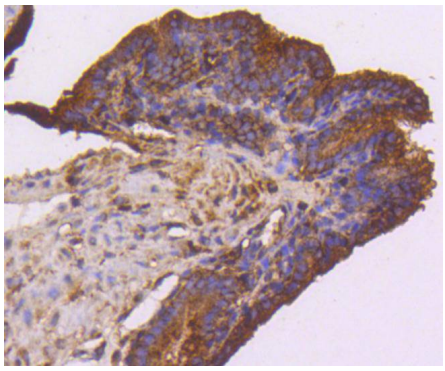

Fig7: Immunohistochemical analysis of paraffin-embedded mouse placenta tissue using anti-VEGF Receptor 1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1605-11, 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.

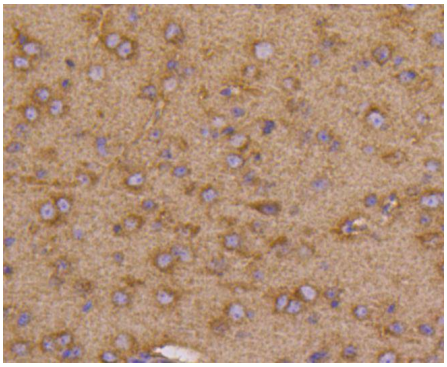

Fig8: Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-VEGF Receptor 1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1605-11, 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.

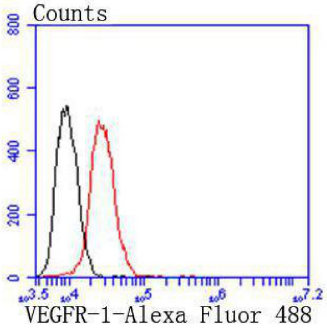

Fig9: Flow cytometric analysis of VEGF Receptor 1 was done on A431 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1605-11, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.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. Zhou W et al. Fibroblast growth factor receptor 1 promotes MG63 cell proliferation and is associated with increased expression of cyclin-dependent kinase 1 in osteosarcoma. Mol Med Rep 13:713-9 (2016).
2. Liu X et al. Impaired VEGF Signaling in Lungs with Hypoplastic Esophageal Atresia and Effects on Branching Morphogenesis. Cell Physiol Biochem 39:385-94 (2016).