Anti-NGF Antibody [SI79-01]

ET1606-29

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IHC-Fr
Molecular Wt:	Predicted band size: 27 kDa
Clone number:	SI79-01

Description: Nerve growth factor (NGF) is a neurotrophic factor and neuropeptide primarily involved in the regulation of growth, maintenance, proliferation, and survival of certain target neurons. It is perhaps the prototypical growth factor, in that it was one of the first to be described. NGF is involved primarily in the growth, as well as the maintenance, proliferation, and survival of nerve cells (neurons). In fact, NGF is critical for the survival and maintenance of sympathetic and sensory neurons, as they undergo apoptosis in its absence. However, several recent studies suggest that NGF is also involved in pathways besides those regulating the life cycle of neurons. NGF can drive the expression of genes such as bcl-2 by binding to the Tropomyosin receptor kinase A, which stimulates the proliferation and survival of the target neuron. There is evidence that pancreatic beta cells express both the TrkA and p75NTR receptors of NGF. It has been shown that the withdrawal of NGF induces apoptosis in pancreatic beta cells, signifying that NGF may play a critical role in the maintenance and survival of pancreatic beta cells. NGF plays a critical role in the regulation of both innate and acquired immunity. In the process of inflammation, NGF is released in high concentrations by mast cells, and induces axonal outgrowth in nearby nociceptive neurons. This leads to increased pain perception in areas under inflammation. In acquired immunity, NGF is produced by the Thymus as well as CD4+ T cell clones, inducing a cascade of maturation of T cells under infection.

Immunogen:	Synthetic peptide within Human NGF aa 192-241 / 241.
Positive control:	HL-60 cell lysate, HeLa cell lysate, MDA-MB-231 cell lysate, human liver tissue lysate, mouse liver tissue lysate, rat liver tissue lysate, mouse skeletal muscle tissue lysate, rat skeletal muscle tissue lysate, NIH/3T3, mouse liver tissue, mouse brain tissue, mouse thymus tissue, rat brain tissue.
Subcellular location:	Secreted.
Database links:	SwissProt: P01138 Human | P01139 Mouse | P25427 Rat
Recommended Dilutions:	
WB	1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:100
IHC-Fr	1:100
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

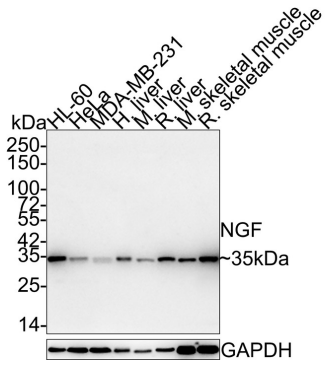

Fig1: Western blot analysis of NGF on different lysates with Rabbit anti-NGF antibody (ET1606-29) at 1/2,000 dilution.

Lane 1: HL-60 cell lysate
Lane 2: HeLa cell lysate
Lane 3: MDA-MB-231 cell lysate
Lane 4: Human liver tissue lysate
Lane 5: Mouse liver tissue lysate
Lane 6: Rat liver tissue lysate
Lane 7: Mouse skeletal muscle tissue lysate
Lane 8: Rat skeletal muscle tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 27 kDa
Observed band size: 35 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 (ET1606-29) at 1/2,000 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.

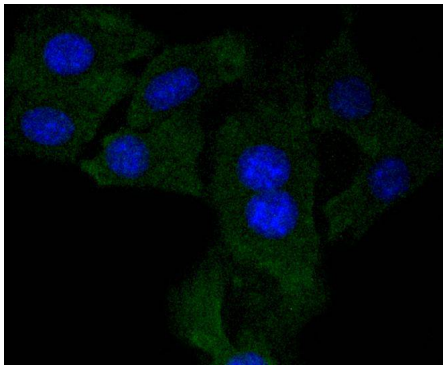

Fig2: ICC staining of NGF in NIH/3T3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1606-29, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

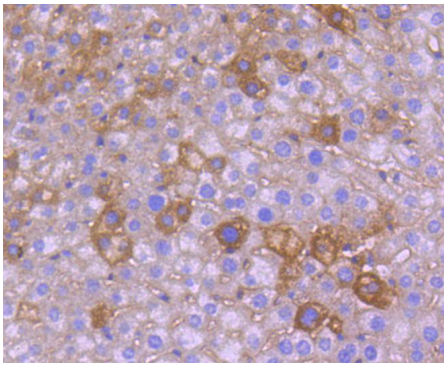

Fig3: Immunohistochemical analysis of paraffin-embedded mouse liver tissue using anti-NGF antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1606-29, 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.

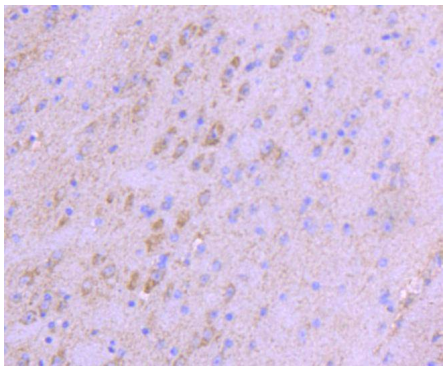

Fig4: Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-NGF antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1606-29, 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.

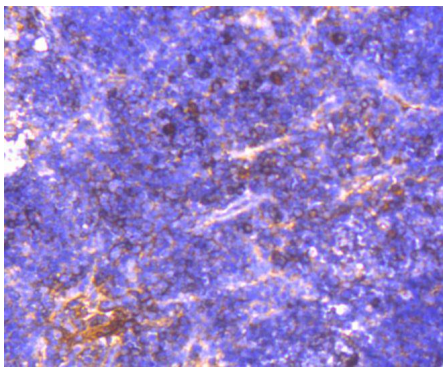

Fig5: Immunohistochemical analysis of paraffin-embedded mouse thymus tissue using anti-NGF antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1606-29, 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.

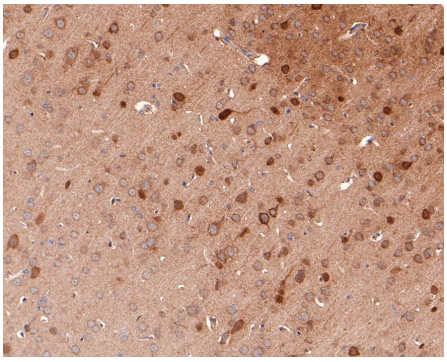

Fig6: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-NGF antibody (ET1606-29) at 1/100 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1606-29) at 1/100 dilution for 1 hour 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.

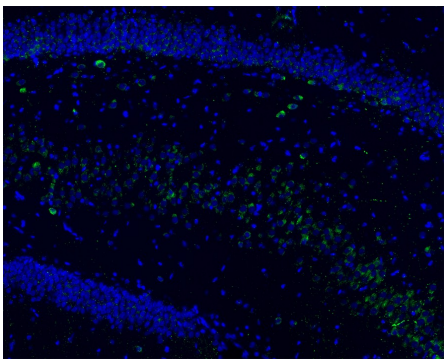

Fig7: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling NGF with Rabbit anti-NGF antibody (ET1606-29).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1606-29, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

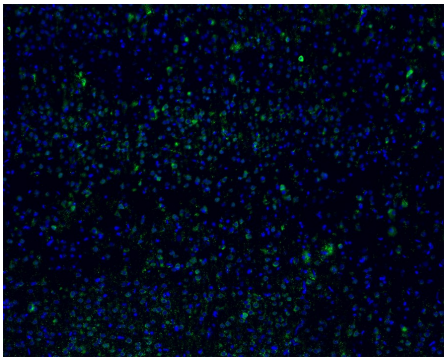

Fig8: Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling NGF with Rabbit anti-NGF antibody (ET1606-29).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1606-29, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

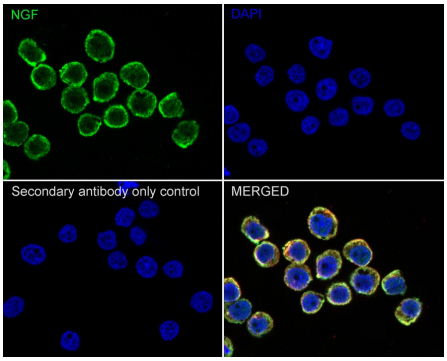

Fig9: Immunocytochemistry analysis of HL-60 cells labeling NGF with Rabbit anti-NGF antibody (ET1606-29) at 1/100 dilution.

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-NGF antibody (ET1606-29) 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.

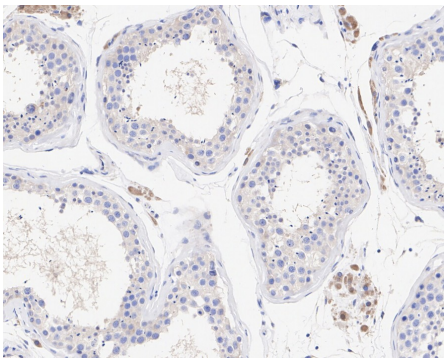

Fig10: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-NGF antibody (ET1606-29) at 1/1,000 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1606-29) at 1/1,000 dilution for 1 hour 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.

Background References

1. Moon HJ et al. Effects of secreted factors in culture medium of annulus fibrosus cells on microvascular endothelial cells: elucidating the possible pathomechanisms of matrix degradation and nerve in-growth in disc degeneration. Osteoarthritis Cartilage 22:344-54 (2014).
2. Vivas O et al. Nerve growth factor sensitizes adult sympathetic neurons to the proinflammatory peptide bradykinin. J Neurosci 34:11959-71 (2014).