Product Datasheet

NEFL/NF-L/NF Rabbit Polyclonal Antibody (orb738390)

Catalog Number	orb738390
Category	Antibodies
Description	Anti-NEFL/NF-L Antibody. Tested in ELISA, Flow Cytometry, IF, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Target	Neurofilament light polypeptide
Clonality	Polyclonal
Species/Host	Rabbit
Isotype	Rabbit IgG
Conjugation	Unconjugated
Reactivity	Human, Mouse, Rat
Form/Appearance	Lyophilized
Concentration	500 µg/ml
Buffer/Preservatives	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.
Purification	Immunogen affinity purified.
Immunogen	E.coli-derived human NEFL/NF-L recombinant protein (Position: F4-A463).
UniProt ID	P07196

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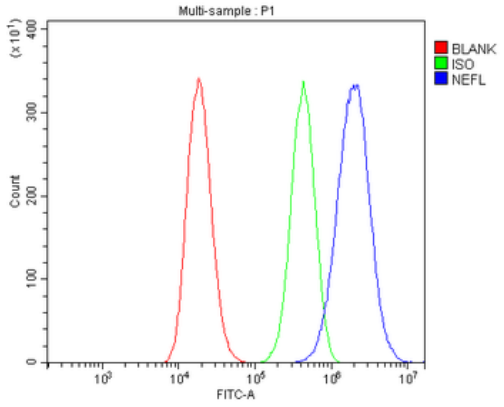
MW	72 kDa
Tested applications	ELISA, FC, IF, IHC, WB
Dilution range	Western blot, 0.25-0.5µg/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5µg/ml, Human, Mouse, Rat Immunofluorescence, 5 µg/ml, Mouse, Rat Flow Cytometry (Fixed), 1-3µg/1x10^6 cells, Human ELISA, 0.1-0.5µg/ml
Cross Reactivity	No cross-reactivity with other proteins.
Antibody Type	Primary Antibody
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Note	For research use only
Expiration Date	12 months from date of receipt.



Flow Cytometry analysis of 293T cells using anti-NEFL/NF-L antibody. Overlay histogram showing 293T cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-NEFL/NF-L Antibody (1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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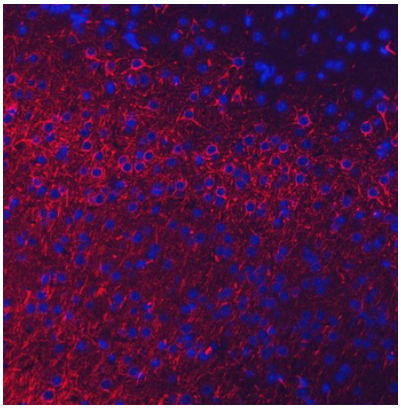
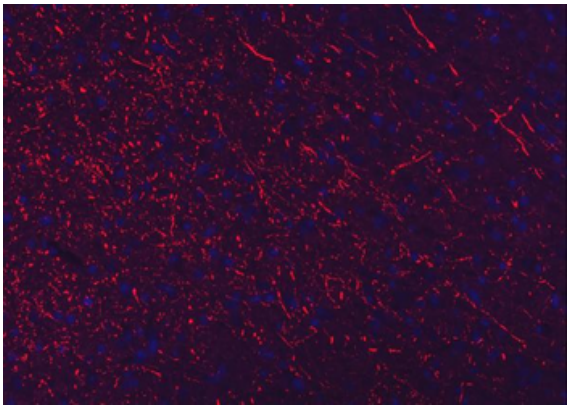
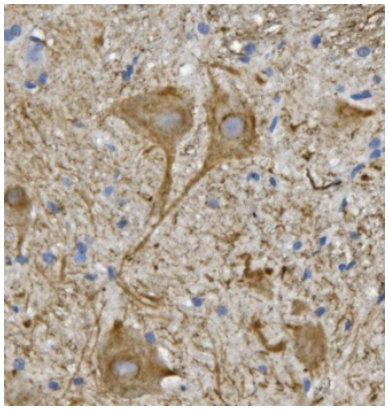


IF analysis of NEFL/NF-L using anti-NEFL/NF-L antibody. NEFL/NF-L was detected in a paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 µg/mL rabbit anti-NEFL/NF-L Antibody overnight at 4°C. Biotin conjugated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Cy3 Conjugated Avidin. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IF analysis of NEFL/NF-L using anti-NEFL/NF-L antibody. NEFL/NF-L was detected in a paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 µg/mL rabbit anti-NEFL/NF-L Antibody overnight at 4°C. Biotin conjugated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Cy3 Conjugated Avidin. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IHC analysis of NEFL/NF-L using anti-NEFL/NF-L antibody. NEFL/NF-L was detected in paraffin-embedded section of mouse spinal cord tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-NEFL/NF-L Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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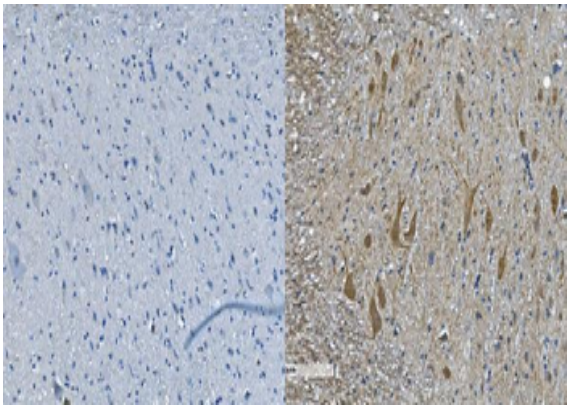
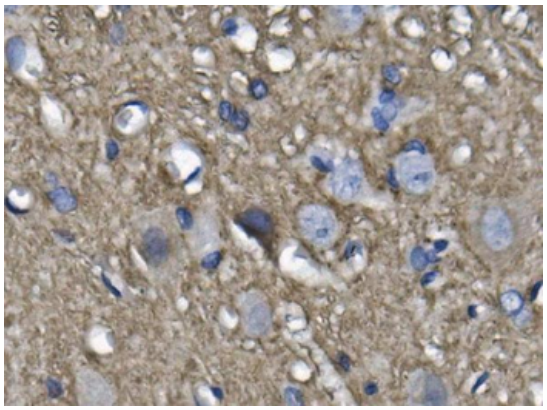


IHC analysis of NEFL/NF-L using anti-NEFL/NF-L antibody. NEFL/NF-L was detected in paraffin-embedded section of mouse spinal cord tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-NEFL/NF-L Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.



IHC analysis of NEFL/NF-L using anti-NEFL/NF-L antibody. NEFL/NF-L was detected in paraffin-embedded section of rat spinal cord tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-NEFL/NF-L Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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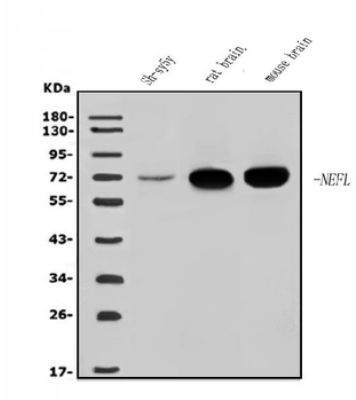

Western blot analysis of NEFL/NF-L using anti-NEFL/NF-L antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50 ug of sample under reducing conditions. Lane 1: human SH-SY5Y whole cell lysates, Lane 2: rat brain tissue lysates, Lane 3: mouse brain tissue lysates. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-NEFL/NF-L antigen affinity purified polyclonal antibody at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1% Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with Tanon 5200 system. A specific band was detected for NEFL/NF-L at approximately 72 KD. The expected band size for NEFL/NF-L is at 72 KD.

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