

## Anti-NEFL/NF-L Antibody Picoband™

Catalog Number: A02482-1

### About NEFL

Neurofilament light polypeptide (NFL), also known as neurofilament light chain, is a neurofilament protein that in humans is encoded by the NEFL gene. Neurofilaments are type IV intermediate filament heteropolymers composed of light, medium, and heavy chains. Neurofilaments comprise the axoskeleton and they functionally maintain the neuronal caliber. They may also play a role in intracellular transport to axons and dendrites. This gene encodes the light chain neurofilament protein. Mutations in this gene cause Charcot-Marie-Tooth disease types 1F (CMT1F) and 2E (CMT2E), disorders of the peripheral nervous system that are characterized by distinct neuropathies. A pseudogene has been identified on chromosome Y.

### Overview

Product Name	Anti-NEFL/NF-L Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-NEFL/NF-L Antibody Picoband™ catalog # A02482-1. Tested in ELISA, Flow Cytometry, IF, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, Flow Cytometry, IF, IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P07196

### Technical Details

Immunogen	E.coli-derived human NEFL/NF-L recombinant protein (Position: F4-A463).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.

**Suggested Dilutions**

Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.

If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.

Some PubMed article(s) citing the expression level of this target are as follows:

Boster Bio's internal QC testing used:

Western blot, 0.25-0.5ug/ml, Human, Mouse, Rat

Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Mouse, Rat

Immunofluorescence, 5 ug/ml, Mouse, Rat

Flow Cytometry, 1-3ug/1x10<sup>6</sup> cells, Human

Direct ELISA, 0.1-0.5ug/ml, Human

## Anti-NEFL/NF-L Antibody Picoband™ (A02482-1) Images

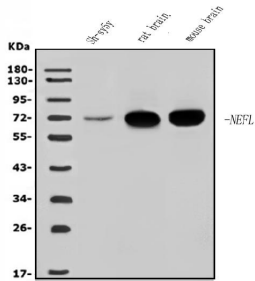


Figure 1. Western blot analysis of NEFL/NF-L using anti-NEFL/NF-L antibody (A02482-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

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 150mA 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 (Catalog # A02482-1) at 0.5 ug/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 (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for NEFL/NF-L at approximately 72KD. The expected band size for NEFL/NF-L is at 72KD.

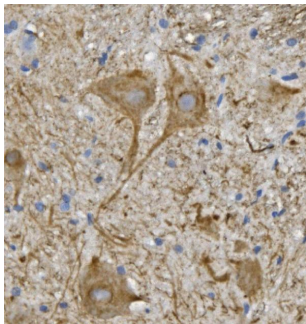


Figure 2. IHC analysis of NEFL/NF-L using anti-NEFL/NF-L antibody (A02482-1).

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 2ug/ml rabbit anti-NEFL/NF-L Antibody (A02482-1) 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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

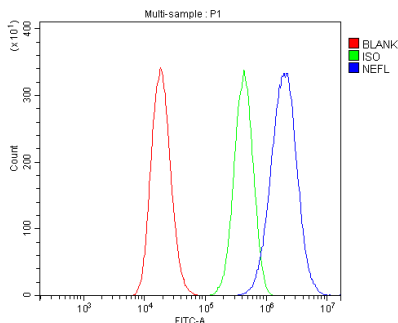
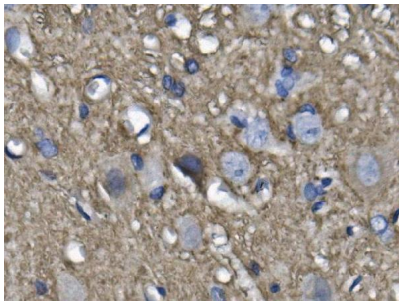


Figure 3. Flow Cytometry analysis of 293T cells using anti-NEFL/NF-L antibody (A02482-1).

Overlay histogram showing 293T cells stained with A02482-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-NEFL/NF-L Antibody (A02482-1, 1ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Figure 4. IHC analysis of NEFL/NF-L using anti-NEFL/NF-L antibody (A02482-1).



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 2ug/ml rabbit anti-NEFL/NF-L Antibody (A02482-1) 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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

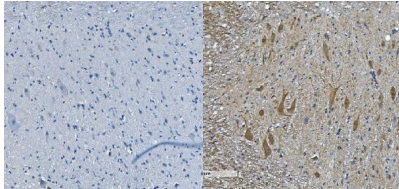


Figure 5. IHC analysis of NEFL/NF-L using anti-NEFL/NF-L antibody (A02482-1). NEFL/NF-L was detected in paraffin-embedded section of mouse spinal cord tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-NEFL/NF-L Antibody (A02482-1) 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 Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

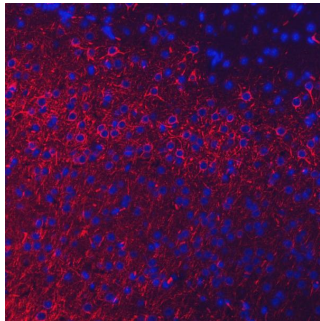


Figure 6. IF analysis of NEFL/NF-L using anti-NEFL/NF-L antibody (A02482-1). NEFL/NF-L was detected in a paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 ug/mL rabbit anti-NEFL/NF-L Antibody (A02482-1) overnight at 4°C. Biotin conjugated goat anti-rabbit IgG (BA1003) was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Cy3 Conjugated Avidin (BA1037). The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

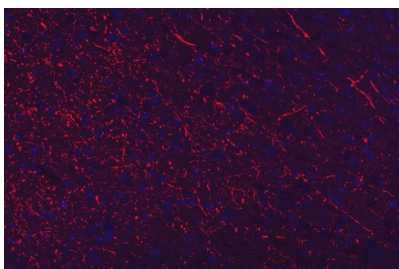


Figure 7. IF analysis of NEFL/NF-L using anti-NEFL/NF-L antibody (A02482-1). NEFL/NF-L was detected in a paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 ug/mL rabbit anti-NEFL/NF-L Antibody (A02482-1) overnight at 4°C. Biotin conjugated goat anti-rabbit IgG (BA1003) was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Cy3 Conjugated Avidin (BA1037). The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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