

Product Datasheet

Nefh Rabbit Polyclonal Antibody (orb738389)

Catalog Number	orb738389
Category	Antibodies
Description	Anti-Nefh Antibody. Tested in ELISA, Flow Cytometry, IHC, WB applications. This antibody reacts with Mouse, Rat.
Target	Neurofilament heavy 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 Na ₂ HPO ₄ .
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.
Purification	Immunogen affinity purified.
Immunogen	E.coli-derived mouse Nefh recombinant protein (Position: Y109-E466).
UniProt ID	P19246
MW	117-220 kDa

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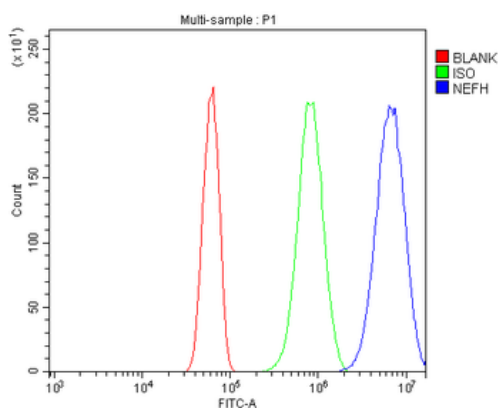
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Tested applications	ELISA, FC, IHC, WB
Dilution range	Western blot, 0.1-0.25µg/ml, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5µg/ml, Human, Mouse, Rat Flow Cytometry (Fixed), 1-3µg/1x10 ⁶ cells, Mouse ELISA, 0.1-0.5µg/ml, -
Cross 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 Neuro-2a cells using anti-Nefh antibody. Overlay histogram showing Neuro-2a 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-Nefh Antibody (1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10⁶) 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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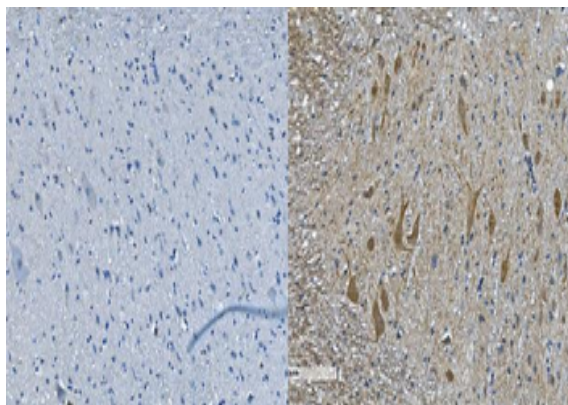
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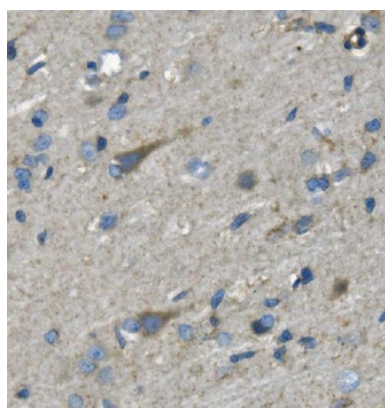
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IHC analysis of Nefh using anti-Nefh antibody. Nefh was detected in paraffin-embedded section of mouse spinal cord tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Nefh 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 Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



IHC analysis of Nefh using anti-Nefh antibody. Nefh was detected in 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 2 µg/ml rabbit anti-Nefh 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 Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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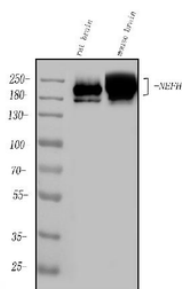
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Western blot analysis of Nefh using anti-Nefh antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50 ug of sample under reducing conditions. Lane 1: rat brain tissue lysates, Lane 2: 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-Nefh antigen affinity purified polyclonal antibody at 0.25 $\mu\text{g}/\text{mL}$ 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 Nefh at approximately 117-220 KD. The expected band size for Nefh is at 117 KD.

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