

BOSTER BIOLOGICAL TECHNOLOGY, LTD.

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www.bosterbio.com

Certificate of Analysis

Note: this is a sample COA. To get the COA for your lot #, please contact us at support@bosterbio.com

To whom it may concern,

This letter attests that Anti-NEFH Antibody (Monoclonal, N52) , catalog # MA1071, is manufactured by Boster Biological Technology., Ltd, through test and assay, we got the following data:

1. Immunogen

C-terminal segment of enzymatically dephosphorylated pig Neurofilament 200.

2. Application Data

Applications Details

Immunohistochemistry(Paraffin-embedded Section), 1-2 μ g/ml, Human, mouse, rat, By Heat
Immunohistochemistry(Frozen Section), 1-2 μ g/ml, Human, mouse, rat, -
Western blot, 0.5ml, Human, mouse, rat

3. Cross Reaction

No cross reactivity with other proteins

4. Image

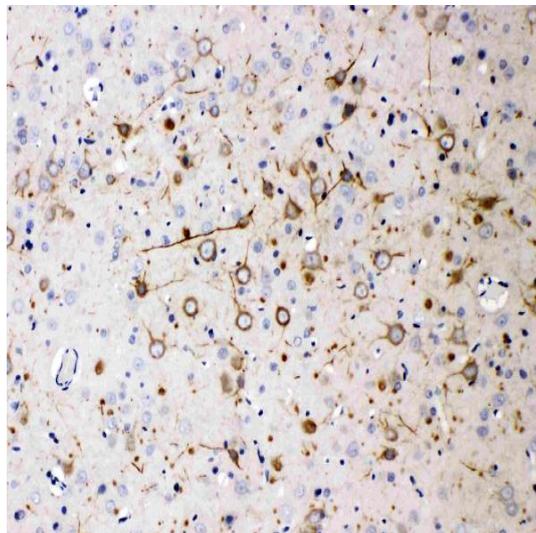


Figure 1. IHC analysis of NEFH using anti- NEFH antibody (MA1071).

NEFH was detected in paraffin-embedded section of rat brain tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml mouse anti- NEFH Antibody (MA1071) overnight at 4 $^{\circ}$ C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37 $^{\circ}$ C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

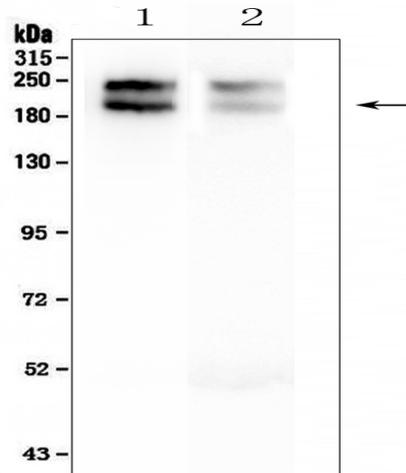


Figure 2. Western blot analysis of NEFH using anti-NEFH antibody (MA1071).

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: rat brain tissue lysates,

Lane 2: 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 mouse anti-NEFH antigen affinity purified monoclonal antibody (Catalog # MA1071)) at 0.5 μ g/mL overnight at 4 $^{\circ}$ C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at

RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for NEFH at approximately 200KD.

The expected band size for NEFH is at 112KD.

Approved by:

Ms. Yangjing Yue Chief technician

Quality Control System of ELISA

Date: May 13, 2016