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Mouse NF-L Ready-To-Use IHC Kit

Cat. No.: IHC0129M
Sample Type: FFPE tissue **Size: 50T (including 1 control slide)**
Storage and Stability: Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

General Information

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8℃
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8℃
4	Blocking Buffer	3 ml	RTU	2-8℃
5	Primary Antibody (Mouse NF-L Rabbit pAb)	6 ml	RTU	2-8℃
6	Secondary Antibody (HRP-Goat anti-Rabbit IgG pAb)	6 ml	RTU	2-8℃
7	Chromogen Component A	0.3 ml	RTU	-20℃
8	Chromogen Component B	0.3 ml	RTU	-20℃
9	Counter Staining Reagent	5 ml	RTU	RT
10	Differentiation Reagent	6 ml	RTU	RT
11	Mounting Media	5 ml	RTU	RT
12	Control slide (Mouse cerebellum)	1 slide	RTU	RT
13	Datasheet	1 copy		

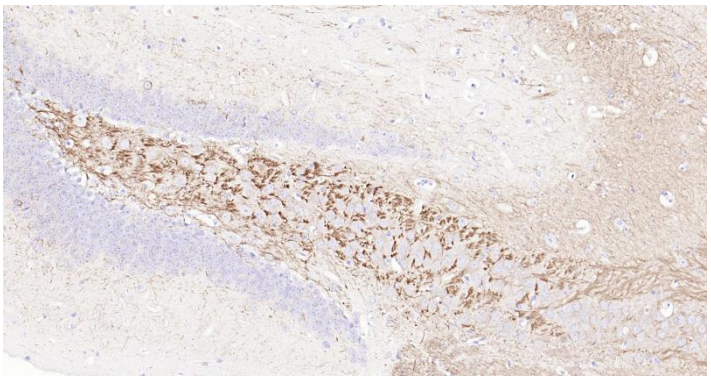
Background

Involved in the maintenance of neuronal caliber, neurofilaments are the intermediate filament proteins found specifically in neurons, and are composed predominantly of three major proteins called NF-L, NF-M and NF-H. Like most other intermediate filament proteins (IFPs), the expression of the different neuronal IFPs is both tissue-specific and developmentally regulated. NF-L is the light or low molecular weight microfilament subunit and runs on SDS-PAGE gels at approximately 70 kDa. Neurofilament are the 10nm or intermediate filament proteins found specifically in neurons, and are composed predominantly of three major proteins called NF-L, NF-M and NF-H. NF-H is the heavy or high molecular weight microfilament subunit and runs on SDS-PAGE gels in the range 180-220 kDa, with some variation in different species.

Synonyms

Neurofilament L; Neurofilament 68; Neurofilament triplet L; 70 kD Neurofilament Light; 68kDa neurofilament protein; CMT 1F; CMT 2E; CMT1F; CMT2E; FLJ53642; Light molecular weight neurofilament protein; NEFL; Neurofilament light; Neurofilament light polypeptide 68kDa; Neurofilament light polypeptide; Neurofilament protein, light chain; Neurofilament subunit NF L; Neurofilament triplet L protein; NF 68; NF L; NF68; NFL; NFL_HUMAN.

Validation Data



Immunohistochemical analysis of paraffin embedded mouse brain tissue slide using IHC0129M (Mouse NF-L IHC Kit).

Immunohistochemistry Protocol

1. Deparaffinization And Rehydration

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. Antigen Retrieval

Add 100×**Antigen Retrieval Buffer** into distilled water to prepare a 1×solution. Boil slides in 1×solution at 95°C-100°C for 15 minutes. Move the slides to 1×solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

3. Block Endogenous Peroxidase

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

4. Serum Blocking

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

5. Primary Antibody Incubation

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **Mouse NF-L Rabbit pAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

6. Secondary Antibody Incubation

Incubate slides with 2-4 drops of **HRP-Goat anti-Rabbit IgG pAb** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

7. Signal Development

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer** (**Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18**) to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

8. **Counterstain**

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. Dehydration Sheet

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, please take necessary precautions.
5. PBS (reagent 1) can be stored for one week at 4℃ after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as " IHC0129M, Bioss Antibodies". Citation example: "Mouse tissue sections using Mouse NF-L IHC Kit (IHC0129M, Bioss Antibodies) were stained for NF-L according to the manufacturer's instructions.