

NF-L Ready-To-Use IHC Kit

Cat.No:	IHC0129
Applications:	IHC-P
Reactivity:	Human, Mouse, Rat
Size:	50T
Assay type:	Immunohistochemistry
Sample type:	FFPE tissue

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°C
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8°C, protect from light
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (NF-L Rabbit pAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (HRP-Goat anti-Rabbit IgG pAb)	6 ml	RTU	2-8°C
7	Chromogen Component A	0.3 ml	RTU	-20°C,protect from light
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (人小脑,大鼠小脑,小鼠小脑)	3 slides	RTU	RT
12	Datasheet	1 copy		

Immunohistoche

mistry 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 $100 \times$ **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 **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°C after preparation; The antigen retrieval buffer ($1 \times$ 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 " IHC0129, Bioss Antibodies". Citation example: " Tissue sections using NEFL IHC Kit (IHC0129, Bioss Antibodies) were stained for NEFL according to the manufacturer's instructions."

Introduction:

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.

Validation Data



Immunohistochemical analysis of paraffin embedded human cerebellum tissue slide using IHC0129 (NF-L IHC Kit).



Immunohistochemical analysis of paraffin embedded rat cerebellum tissue slide using IHC0129 (NF-L IHC Kit).



Immunohistochemical analysis of paraffin embedded mouse spinal cord tissue slide using IHC0129 (NF-L IHC Kit).



Immunohistochemical analysis of paraffin embedded rat spinal cord tissue slide using IHC0129 (NF-L IHC Kit).



Immunohistochemical analysis of paraffin embedded mouse cerebellum tissue slide using IHC0129 (NF-L IHC Kit).