Neurofilament (ABT208) IHC Kit IHC Kit

Catalog Number

IHCM6895-3mL

IHCM6895-6mL

IHCM6895-10mL

Intended use

For Research Use Only. Not for use in diagnostic procedures.

Application:

Immunohistochemistry

Introduction

Neurofilament (NF) is a neuron specific intermediate filament protein. It is a polymer composed of subunits of NF-H, NF-M and NF-L. It exists in neurons, neurites, peripheral nerve fibers, sympathetic ganglion cells and adrenal medulla. It is expressed in ganglioneuroma, paraganglioma, cerebellar or peripheral neuroblastoma, adrenal tumor or peripheral pheochromocytoma. It is helpful to the diagnosis and differential diagnosis of neuroblastoma and pheochromocytoma, and to identify the neurons of Hirschsprung's disease.

Principle of Procedure

A specific primary antibody targeting the antigen is applied to the tissue section, followed by several horseradish peroxidase (HRP)-conjugated anti-mouse/rabbit IgG polymer molecules that bind to the primary antibody, forming an immune complex. The HRP catalyzes the chromogenic substrate 3,3'-diaminobenzidine (DAB), generating an insoluble brown precipitate that marks the localization of the target antigen, allowing visualization under a microscope.

Reagent provided

Component Description	Component Cat. No	IHCM6895-3mL	IHCM6895-6mL	IHCM6895-10mL	Storage
Neurofilament (ABT208) Mouse mAb (Ready to Use)	YM6895R	3mL	6mL	10mL	2-8°C
PathoBlocker™-2in1 Blocking Buffer	IHCX010	3mL	6mL	10mL	2-8°C
Negative control antibody (prediluted)	VN003R	3mL	6mL	10mL	2-8°C
Immunohistochemistry Enhancer (Mouse)	RS0052	3mL	6mL	10mL	2-8°C
PolyVision™ Polymer HRP Goat Anti Mouse/Rabbit IgG(H+L)-S	RS0011	3mL	6mL	10mL	2-8°C
Buffer Stock Solution	IHCX007	3mL	6mL	10mL	2-8°C
DAB Stock Solution					
Hydrogen Peroxide Stock Solution					
DAB preparation bottle		1 bottle	1 bottle	1 bottle	2-8°C

Storage

Store at 2-8° C. Do not freeze.

Materials required but not provided

The materials required, but not provided with the primary antibody are:

1. Positive and negative control tissue
2. Microscope slides, positively charged
3. Drying oven capable of maintaining a temperature of 53-65°C
4. Staining jars
5. Timer
6. Xylene or xylene substitute, such as Patho3in1™-Low pH (Cat. No. IHCX001) or Patho3in1™-High pH (Cat. No. IHCX004)
7. Ethanol or reagent alcohol
8. Antigen Retrieval Solution, such as Patho3in1™-Low pH (Cat. No. IHCX001) or Patho3in1™-High pH (Cat. No. IHCX004)
9. Deionized or distilled water
10. Heating equipment, such as a water bath or electric pressure cooker
11. Antibody diluents, such as Aurora Green Diluent (Cat. No. IHCX012), Twilight Blue Diluent (Cat. No. IHCX013), Sunrise Red Diluent (Cat. No. IHCX014), Sunset Yellow Diluent (Cat. No. IHCX015),
12. PBST, such as IHC Wash Buffer (Cat. No. IHCX017)

13. Hematoxylin Counterstaining Solution (Cat. No. IHCX018)
14. Clear reagent, such as Xylene or PathoClear™ Dewaxing Reagent (Cat. No. IHCX019)
15. Mounting Medium (Cat. No. IHCX020)
16. Cover glass
17. Light microscope (40-400x)

Precautions

1. Professional use only.
2. Wear appropriate Personal Protective Equipment when handling reagents to avoid contact with eyes, skin and mucous membranes. If contact occurred, wash the contacted area with copious amounts of water immediately.
3. This product contains Proclin 300, a chemical classified as an irritant and may cause sensitization through skin contact. Use protective clothing and gloves when handling.
4. Use proper procedures when handling products derived from biological sources.
5. Avoid microbial contamination which could lead to incorrect results.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of according to local, State and Federal regulations.
7. Consult local authorities when dispose of unused solution.

Specimen preparation

This product is suitable for labeling paraffin-embedded, formalin-fixed tissue sections. Before IHC staining, the tissue samples should be processed. The recommended tissue process is fixation, dehydration, clearing, infiltration of paraffin, and embedding and sectioning of tissues. The tissue sections should be placed on a positively charged glass slide, baked 2 hours in a 60-65°C oven and prepared for staining.

Staining procedure

Due to variation in specimen type, prepare processing, lab instrument and environmental conditions, the optimal performance could be achieved by each laboratory's optimized manual. The user must validate results obtained with this antibody, especially when utilized with other staining systems or automated platforms. Avoid tissue sections from drying out through the entire procedure.

Protocol

3-Step Procedure

1. Deparaffinization: Place the sections on a slide rack and place the rack in a 60–65°C oven for 1 hour. Then transfer the rack to a xylene bath and perform two xylene baths, 10 minutes each.
2. Rehydration: Rehydrate the sections in 100%, 100%, 95%, and 85% ethanol for 5 minutes each. Wash the slides gently in running tap water for 30 seconds. Then immerse the sections in distilled water 5 times. Place the sections in distilled water for further use.
3. Heat-induced epitope retrieval: Various heating methods may be used; two recommended methods are as follows:

   Water bath method: Pour enough Antigen Retrieval Solution into a staining tank so that all tissues on the slides are submerged, heat the buffer to 98°C, then place the slide rack into the staining tank and incubate the slides in the buffer for 20 minutes. Wait for the buffer to cool to room temperature, then transfer the sections to distilled water and immerse them in distilled water 5 times. Place the sections in distilled water for further use.

   Pressure cooker method: Pour an appropriate amount of water into the pressure cooker and place the staining tank (with enough Antigen Retrieval Solution) inside the cooker. Heat the buffer to boiling without securing the lid. Once boiling, transfer the slides to the staining tank, secure the lid, and as soon as the cooker reaches full pressure, keep it at pressure for 3 minutes. Then stop heating and activate the pressure release valve. Open the lid after the cooker has depressurized. Wait for the buffer to cool to room temperature, then transfer the sections to distilled water and immerse them in distilled water 5 times. Place the sections in distilled water for further use.
4. Block endogenous peroxidase activity: Use a PAP pen to draw a hydrophobic circle around the tissue. Apply the PathoBlocker™-2in1 Blocking Buffer provided in the kit to cover the tissue sections. Incubate for 10 minutes at room temperature to quench endogenous peroxidase activity. Rinse the slides and immerse the sections in PBS buffer 5 times.
5. Primary antibody incubation: Apply an appropriate volume of the ready-to-use primary antibody to cover the tissue section. Meanwhile, apply the prediluted negative control antibody provided in the kit to the tissue section as required by the experiment. Incubate in a humidified chamber for 30 minutes at room temperature or overnight at 4°C.
6. Rinse the slides with PBST and place the sections in a staining rack. Then immerse them in PBST buffer 4 times, 5 minutes each.
7. Enhancer incubation (Optional): Apply the Immunohistochemistry Enhancer (Mouse) provided with the kit to cover the tissue sections. Incubate in a humidified chamber for 20 minutes at room temperature.
8. Rinse the slides with PBST and place the sections in a staining rack. Then immerse them in PBST buffer 4 times, 5 minutes each.
9. Secondary antibody incubation: Arrange the slides on a staining tray. Rinse off the PBST buffer and wipe the slides. Apply the PolyVision™ Polymer HRP Goat Anti Mouse/Rabbit IgG provided with the kit. Incubate the tissue slides in a humidified chamber for 30 minutes at room temperature.
10. Rinse the slides with PBST buffer, then place them in a staining rack and immerse in PBST buffer 4 times, 5 minutes each.
11. Chromogenic reaction:

    DAB Chromogen Solution Preparation: To prepare 1 mL of DAB working solution, add 20 µL of Buffer Stock Solution, 20 µL of DAB Stock Solution, 20 µL of Hydrogen Peroxide Stock Solution, and 940 µL of distilled water.

    DAB Chromogen Application: Arrange the slides on a staining tray, drain the PBST, and wipe the slides. Add the DAB working solution to cover the tissue and observe the color development by eye or under a light microscope. Once the desired color intensity is reached, terminate the reaction by rinsing the slides with distilled water, immersing them in distilled water again, and washing the slides in running tap water for 2–5 minutes.
12. Counterstain: Immerse slides in hematoxylin for 30 seconds to 5 minutes (depending on hematoxylin preparation time; 30 seconds for freshly prepared hematoxylin solution). Wash the slides in running tap water for 2–5 minutes.
13. Dehydration: Dehydrate the tissue slides sequentially through 85% alcohol for 5 minutes, 95% alcohol for 5 minutes, and 100% alcohol for 5 minutes (twice)

14. Clearing: When the tissue slides are dried, clear the tissue in xylene or a xylene substitute for 2–5 minutes.

15. Mounting: Use mounting medium to mount the sections and avoid air bubbles. Remove excess mounting solution and visualize the result under a microscope.

3in1 Procedure

1. Deparaffinization, Rehydration, Heat-induced epitope retrieval:

Water bath method: Place the slides on a slide rack and place the rack in a 60–65°C oven for 1 hour. Prepare two staining tanks and pour enough Patho3in1™ Solution into the two staining tank so that all tissues on the slides are submerged. The second tank filled with Patho3in1™ Solution will be used as a hot rinse following processing time in the first staining tank. Heat the Patho3in1™ Solution to 98°C and place the slide rack into the first staining tank and incubate the slides in the buffer for 20 minutes. Then carefully transfer the slides from the first container to the second tank using forceps, gradually lower the slide rack into the heated rinse solution in a controlled manner. Transfer the slides to PBS buffer (1X PBS powder is provided with Patho3in1™ Solution) and immerse them in PBS buffer 5 times. Place the slides in PBS buffer for further use.

Pressure cooker method: Place the slides on a slide rack and place the rack in a 60–65°C oven for 1 hour. Prepare two staining tanks and pour enough Patho3in1™ Solution into the two staining tanks so that all tissues on the slides are submerged. The second tank filled with Patho3in1™ Solution will be used as a hot rinse following processing time in the first staining tank. Pour an appropriate amount of water into the pressure cooker and place the two staining tanks inside the cooker. Place the slide rack into the first staining tank and lock pressure cooker lid, and as soon as the cooker reaches full pressure, keep it at pressure for 3-5 minutes. Then stop heating and activate the pressure release valve. Open the lid after the cooker has depressurized. Wait for the buffer to cool to room temperature, then transfer the slides to PBS buffer (1X PBS powder is provided with Patho3in1™ Solution) and immerse them in PBS buffer 5 times. Place the slides in PBS buffer for further use.

Caution: Rapid immersion of the slide rack may cause the hot rinse solution to boil suddenly due to overheating of the solution.

2. Proceed with IHC staining as described in the 3-Step procedure.

Limitations

1. This product is for research use only.

2. This product is only intended for use in IHC.

3. Nonspecific staining with HRP may exhibited when staining with the tissue specimens from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) .

4. Normal sera, if used as blocking buffer, may cause false negative or false positive results due to the presence of auto-antibodies or natural antibodies.

5. Excessive or incomplete counterstaining may interfere proper interpretation of results and should be avoided.

6. A negative result of IHC test, which may be caused by loss of antigen in tissue during the preparation process, insufficient sensitivity of detection method or other factors, only means the antigen fail to be detected, not that the antigen is unexpressed in the tissue assayed.

7. Researchers often ignore that the perfect presentation of IHC results is not only dependent on the staining process, but the quality of tissue specimen is closely related to the staining results. Improper fixation, dehydration, embedding, sectioning and deparaffinization may result in false negative or false positive results.

8. The proper interpretation of the IHC results should be accomplished by a qualified pathologist who is familiar with all the procedures of IHC staining. Also, it requires considerations from various aspects, such as morphological studies, other diagnostic tests and results of controls.

9. This product may present unexpected results in previously tested or untested tissues due to biological variability of antigen expression in pathological tissues.

10. Heat induced epitope retrieval may result in retrieval of unexpected or undesired sites.

Troubleshooting

1. If the positive tissue control exhibits weaker staining than expected or no staining, check other positive controls used on the same run to identify the underlying causes related to the primary antibody or other reagents, such as reagent omitted, used in wrong order, excessively diluted, defective after expiration date, or incorrect preparation of substrate-chromogen mixture. It may also be caused by tissues improperly collected, fixed or dewaxed.
2. If background is seen in both control tissues and specimen tissues, it may be caused by excessive incubation with substrate-chromogen reagent, secondary antibody cross-reacts with antigens, slides inadequately rinsed, or incomplete removal of paraffin.
3. False-positive results may be presented if tissue proteins non-immunological bind with antibody. It may also be caused by incompletely quenched endogenous biotin and enzymes.

Contact information

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