Anti-Human Neuron-Specific Enolase
Mouse Monoclonal antibody

Clone ABT209

Product Identification

Catalog number	Description
YM6755	concentrate
YM6755R	predilute ready-to-use

Use

Anti-Human Neuron-Specific Enolase (clone ABT209), is used for scientific research in immunohistochemistry (IHC). The antibody is directed against human Neuron-Specific Enolase, exhibiting a cell cytoplasmic staining pattern. The antibody is used for qualitative detection of antigens in formalin fixed, paraffin-embedded tissues.

Introduction

Neuron-specific enolase (NSE) has α, β, γ three subunits, mainly distributed in central nerve, peripheral nerve, neuroendocrine cells and tumor cells, can be used in the auxiliary diagnosis of neuroendocrine tumors. Some non neuroendocrine cells also express NSE, such as normal smooth muscle, myoepithelial cells, renal tubular cells, lymphocytes and so on. It should be combined with other antibodies in the study of neuroendocrine tumors.

Principles and procedures

IHC is a technique that employs antibodies to visualize antigen of interest in tissues. The main principles and procedures are:

1. Fixation of tissues to maintain tissue structure and retain antigenicity.
2. Perform antigen retrieval procedure to re-open the cross-linked epitopes.
3. Sequential application of a specific primary antibody to the antigen, a HRP (Horseradish Peroxidase) labelled polymer secondary antibody to the primary antibody, a chromogenic substrate for HRP, and the HRP catalyze the chromogenic substrate to produce colored precipitation at the antibody-antigen binding site.
4. Sections were counterstained, coverslipped and visualized with a light microscope.

Reagent provided

The concentrated mouse antibody was purified by affinity chromatography using immobilized human Neuron-Specific Enolase peptide and provided in liquid form in phosphate buffered saline, 50% glycerol, 0.05% Proclin 300, and 0.05% BSA. The prediluted ready-to-use antibody is diluted in phosphate buffer saline containing stabilizing protein and 0.05% Proclin 300, and it has been optimized for ready to use; no further dilution is required.

Clone: ABT209. Isotype: IgG1, Kappa.

Mouse IgG concentration: see label on vial.

The concentrations of concentrated antibody may vary between lots, but it does not affect the optimal dilution. Reproducibility studies of inter-lot and lot-to-lot have been conducted to ensure consistent staining results.

Materials required but not provided

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

1. Equipment: drying oven, heating equipment, light microscope.
2. Chemicals: xylene or xylene substitute, ethanol, distilled water, HRP labeled polymer, chromogen, wash buffer, counterstain, antibody diluents, peroxide block, and negative control reagent.
3. Ancillary items: positive and negative control tissue, coverslips, staining rack and staining tray, timer, mounting medium and positively charged slides.

Immunogen

The immunogen is a synthetic peptide corresponding to the human Neuron-Specific Enolase protein.

Specificity

The antibody can specifically recognize human NSE protein.

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.

Storage

Concentrate: store at -20 ℃, do not use after expiration date stamped on vial.

Ready-to-use: store at 2-8 ℃, do not use after expiration date stamped on vial.

It is recommended that controls should always be run simultaneously with test specimens to evaluate the performance of the product. Contact ImmunoWay Biotechnology Company for technical support if there is a suspected problem with the product.

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 ℃ 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.

A simplified protocol for this primary antibody:

1. Dilution: The concentrated antibody can be diluted at a range of 1:200-400 (Antibody Diluent, cat. #YS0001).The prediluted ready-to-use antibody has been optimized for ready to use, no further dilution is required.
2. Epitope Retrieval Technique: heat-induced epitope retrieval. Retrieval solution: 10 mM Tris buffer, 1 mM EDTA, pH 9.0. Retrieval time: 20 minutes.
3. Primary antibody incubation time: 30 minutes incubation at room temperature.
4. Labelled polymer HRP system: apply commercially available detection kit containing peroxidase labelled polymer conjugated to secondary antibodies, refer to the instructions for recommended procedures.
5. HRP-compatible chromogen: according to manufacturer's recommendations.
6. Counterstain and coverslip.

Protocol

A detailed manual protocol of paraffin-embedded, formalin-fixed tissue sections preparation (step 1-step 6) and staining (step 7-step 21) is provided, but it is a guideline only, the optimal conditions should be determined and validated according to the detection method used by each laboratory. All of the following steps were performed at room temperature, quality control (refer to Quality control section) should always be ran simultaneously.

1. Fixation: dissect fresh tissues to less than 3 mm thick and fix with 10% formalin for 24-48 hours. Rinse the tissue with running tap water for 1 hour to clean the fixatives.
2. Dehydration: sequential application of 70%, 80%, and 95% alcohol, 45 minutes each to dehydrate the tissue, which followed by 100% alcohol three times for 1 hour each.
3. Clear the tissue through with xylene 2X for 1 hour each.
4. Immerse the tissue in paraffin 3X for 1 hour each to infiltrate the tissue.
5. Embedding: embed the tissue in paraffin.
6. Section: cut the embedded tissue at 3-5 μm thickness on a microtome and float the tissue sections in a 40 ℃ distilled water bath. Transfer the sections onto positively charged glass slide, baked 2-24 hours in a 60-65 ℃ oven. The sections can be stored at room temperature for up to several years.
7. Deparaffinization: arrange the sections onto a staining rack and place the rack in a 60-65 ℃ oven for 1 hour. Then transfer it to a xylene bath and perform xylene bath two times, 10 minutes each.
8. 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 for five times, 5 minutes each time. Place the sections in distilled water for further use.
9. Heat-induced epitope retrieval: if the retrieval is performed in water bath, pour the retrieval buffer (10 mM Tris, 1 mM EDTA, pH 9.0) into a container, heat the buffer to 98 ℃, and incubate the slides in the buffer for 20 minutes. If it is performed using a pressure cooker, the buffer should be heated to boiling without securing the lid of the pressure cooker. Once boiling, transfer the slides to the cooker, secured the lid of the pressure cooker, and as soon as the cooker has reached full pressure, keep 3 minutes, then stop heating, and activate the pressure release valve. Open the lid after the cooker has been de-pressurized. Wait for the buffer cool to room temperature, transfer the sections to distilled water, and immerse the sections in distilled water for 5X, 5 minutes each.
10. Block endogenous peroxidase activity: immerse the sections in 0.3% H_2O_2 solution, and incubate for 10 minutes to quench endogenous peroxidase activity. Drain the slides and immerse the sections in distilled water for 5X, 3 minutes each.
11. Place the sections on a staining tray. Use a barrier pen to draw a hydrophobic circle around the tissue. Optionally, the slides could be placed in PBST buffer (10 mM PBS, 0.1% tween-20) after drawing to prevent drying out.
12. Primary antibody incubation: dilute concentrated antibody at a range of 1:200-400 (Antibody Diluent, cat. #YS0001).Prediluted ready-to-use antibody could be used immediately. Drain the buffer and wipe slides, add appropriate volume of diluted antibody to cover the tissue section, and incubated in a humidified chamber for 30 minutes.
13. Rinse the slides with PBST and put the sections in a staining rack. Then immerse it in PBST buffer for 4X, 5 minutes each.
14. Secondary antibody incubation: arrange the slides on a staining tray. Drain the PBST buffer and wipe slides. Then cover the tissue with HRP labeled secondary antibody and incubate the tissue slides in a humidified chamber. Refer to the secondary antibody manufacturer's recommendation for incubation temperature and time.
15. Rinse the slides with PBST buffer then put them in a staining rack, and immerse it in PBST buffer for 4X, 5 minutes each.
16. Chromogenic reaction: prepare fresh HRP-compatible chromogen solution such as DAB, AEC according to manufacturer's recommendations before use. Arrange the slides on a staining tray then drain the PBST and wipe slides. Add the chromogen solution to cover the tissue and observe the color development by eyes or under a light microscope. When the desired color intensity is reached, end the reaction by first rinsing the slides with distilled water, then immerse the slides again in distilled water, and wash the slides in running tap water for 2-5 minutes.
17. Counterstain: immerse slides in hematoxylin for 30 seconds - 5 minutes (immerse time is according to the preparation time of hematoxylin. It is 30 seconds for newly prepared hematoxylin solution.). Wash the slides in running tap water for 2-5 minutes.
18. Dehydration: dehydrate the tissue slides through 85% alcohol for 2 minutes, 95% alcohol for 2 minutes, 100% alcohol for 2 minutes, 100% alcohol for 4 minutes.
19. When the tissue slides are dried, clear the tissue in xylene for 1-2 minutes.
20. Use mounting medium to mount the sections and avoid air bubbles.
21. Remove excess mounting solution on the sections and visualize the result under a microscope.

Quality control

Quality control needs to be performed to ensure the stability and repeatability of the performance of this product. Quality control, including positive control tissue, negative control tissue and negative control reagent run simultaneously with the test specimens on the same test run.

Positive Control Tissue

Positive control tissue is a tissue which has been demonstrated to express the target antigen and be used to prevent false negative results. The perfect positive control tissue should be processed in the same manner as the test samples and give weak positive staining. It would be sensitive to the minor changes of the primary antibody. The specific staining result of positive control tissue indicates the proper fixed and processed tissues, correct performance of reagents and proper staining techniques. Using commercially available tissue slides is a second-best choice as these slides were fixed or processed differently from the test specimen, which cannot provide control for the fixation and tissue processing procedure. The result of the test specimen should be considered invalid if the positive control tissue showed negative or weaker staining than expected.

Examples of positive control tissues for this antibody may include the following:

Positive control tissue	
Tissue	Subcellular location
Appendix/ Colon	Cytoplasmic

Negative Control Tissue

Negative control tissue is a tissue which has been demonstrated negative for the tested antigen. It could be used to verify the specificity of antibody and indicate the background staining. The negative control tissue should be processed in the same manner as the test samples and be included in every test run. Refer to the Performance characteristics section for proper negative control tissue. Alternatively, different cell types present in most positive tissues may be considered as internal negative control, but this must be verified by the user. If there is any specific staining occurs in negative control tissue, the results should be considered invalid.

Negative Control Reagent

A negative control reagent is important for detecting nonspecific staining, which allows for better interpretation of staining results. An antibody which is non-human reactive, the same host species and IgG concentration as the primary antibody, could be acted as an ideal negative control reagent. The recommended negative control reagent for this antibody is ImmunoWay Biotechnology Company Mouse IgG1, cat. #VN0001, and should be diluted to the same concentration in the same solution as the primary antibody before use.

Interpretation of results

Positive Control Tissue

The staining results of the positive control tissue should be evaluated first, so it verified all procedures and reagents are working properly. Positive staining result is indicated by an appropriately colored reaction product within the target cells and shows specific cellular staining pattern. The staining could be distributed diffusely or occur as punctate or other characteristics. The location could be nuclear, cytoplasm or cytomembrane according to the antigen tested. The color of the staining is different depending on the substrate chromogens used: red color of AEC (3-amino-9-ethylcarbazole) or brown of DAB (3,3'-diaminobenzidine tetrahydrochloride). Counterstain the tissue with hematoxylin, the cell nuclei will presented as blue color, which gives better contrast to the DAB or AEC staining. Excessive or incomplete counterstaining may interfere proper interpretation of results should be avoided. The result of the test specimen should be considered invalid if the positive control tissue showed negative or weaker staining than expected.

Negative Control Tissue

The staining result of negative control tissue should be examined follow behind the positive control tissue, which could verify the specificity of the primary antibody. No specific staining of negative control tissue demonstrated good specificity of the antibody. Once specific staining occurs, the result of the test specimen should be considered invalid. Counterstain the negative control tissue with hematoxylin - the cell nuclei will presented as blue-purple color. Nonspecific staining caused by necrotic or degenerated cells, cross-linked of antibody, and protein in tissues may presented as a diffuse appearance which is different from specific staining.

Test Tissue

The staining results of test tissue specimens should be evaluated at last. Within the context of background staining indicated by negative reagent control, the positive staining intensity of test tissue specimens should be analyzed. 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. Using a panel of antibodies may be helpful for identifying false negative reactions. A hematoxylin and eosin stained section, which demonstrate the morphology of the tissue sample, should be used to aid in the interpretation of IHC result.

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.

Performance characteristics

Staining tests in normal tissues and abnormal tissues were conducted and the results are listed in the following tables:

Normal tissues

Tissue	# positive / total cases	Tissue	# positive / total cases
Adrenal gland	3/3	Pancreas	2/2
Appendix	2/2	Placenta	2/2
Bladder	1/1	Prostate	0/3
Breast	1/1	Salivary gland	0/1
Cardiac muscle	1/1	Skeletal muscle	0/1
Cerebrum	3/3	Smooth muscle	1/1
Cervix	2/2	Spleen	3/3
Colon	0/3	Stomach	0/3
Esophagus	1/1	Testis	1/1
Kidney	3/3	Thymus	1/1
Liver	0/3	Thyroid	1/1
Lung	2/2	Tonsil	2/2

Abnormal tissues

Disease Tissue	# positive / total cases
Adrenal adenoma	1/1
Bladder carcinoma	3/3
Breast carcinoma	2/3
Cervical squamous cell carcinoma	2/2
Colorectal carcinoma	5/9
Endometrial carcinoma	3/4
Esophageal squamous cell carcinoma	2/2
Gastric adenocarcinoma	1/3
Hepatic carcinoma	0/3
Lung cancer	6/6
Malignant melanoma	0/2
Ovarian carcinoma	2/3
Pancreatic cancer	2/2
Prostatic adenocarcinoma	3/3
Renal clear cell carcinoma	1/1
Thyroid cancer	2/2

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.
4. When used in a given staining system, the improper concentration of primary antibody, too high or too low, may result in false-negative staining.

Contact information

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