Nucleophosmin (ABT210)

PathoELITE™ Mouse Monoclonal antibody

Product Identification

Catalog number	Description
YM6898	Concentrate
YM6898R	Ready-to-Use

Intended use

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

Nucleophosmin (ABT210) Mouse Monoclonal Antibodyis used for scientific research in immunohistochemistry (IHC). The antibody is directed against Nucleophosmin protein, exhibiting a cell nuclear, cytoplasmicstaining. The antibody is used for qualitative detection of antigens in formalin fixed, paraffin-embedded tissues.

Introduction

Nucleophosmin is the most abundant nuclear phosphoprotein in cells, which usually accumulates in the cytoplasm. Generally speaking, the content of tumor growing cells is higher than that of normal quiescent cells. It can be used as a nuclear marker, as well as for the observation of the efficacy of anticancer drugs and the study of cell apoptosis.

Storage

Concentrate: Store at -20°C.

Ready-to-use: Store at 2-8° C. Do not freeze.

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, cover slipped and visualized with a light microscope.

Reagent provided

The concentrated mouse antibody is provided in liquid form in phosphate-buffered saline with 50% glycerol, 0.05% BSA, and 0.05% Proclin 300. The recommended working dilution range is 1:200-400.

The ready-to-use antibody is diluted in phosphate-buffered saline containing stabilizing protein and 0.05% Proclin 300. It has been optimized for direct use, requiring no further dilution.

Isotype: Mouse IgG2b, kappa..

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. Peroxide Block, such as PathoBlocker™-No Serum Blocking Buffer (Cat. No. IHCX011)
12. 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)
13. Negative control antibody, such as Negative Control for Mouse IgG2b, kappa Primary Antibody, Cat.VN003.
14. Detection system, such as PolyVision™ Polymer HRP Goat Anti Mouse/Rabbit IgG(H+L)-S (Cat. No. RS0011) or PolyVision™ Polymer HRP Goat Anti Mouse IgG(H+L)-S (Cat. No. RS0009)
15. Chromogen, such as PathoDAB™-Gold (Cat. No. IHCX007)
16. PBST, such as Wash Buffer (Cat. No. IHCX017)
17. Hematoxylin (Cat. No. IHCX018)

18. Clear reagent, such as Xylene or Patho™Clear (Cat. No. IHCX019)
19. Mounting Medium (Cat. No. IHCX020)
20. Cover glass
21. Light microscope (40-400x)

Immunogen

The immunogen is a synthetic peptide corresponding to the amino acid region 100-200 of the human Nucleophosmin protein..

Specificity

The antibody can specifically recognize human Nucleophosmin 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.

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. Place the sections on a staining tray. Use a PAP pen to draw a hydrophobic circle around the tissue. Optionally, place the slides in distilled water after drawing to prevent drying out.
5. Block endogenous peroxidase activity: Apply the Blocking Buffer to cover the tissue sections or immerse the sections in Blocking Buffer. Incubate for 10 minutes at room temperature to quench endogenous peroxidase activity. Rinse the slides and immerse the sections in PBS buffer 5 times.
6. Primary antibody incubation:

   Concentrate: Dilute the concentrated antibody at a ratio of 1:200-400 with Antibody Diluent. Apply an appropriate volume of the diluted antibody to cover the tissue section. Meanwhile, apply the negative control antibody to the tissue section according to the experiment. Incubate in a humidified chamber for 30 minutes at room temperature or overnight at 4° C.

   Ready-to-Use: Apply an appropriate volume of the ready-to-use antibody to cover the tissue section. Incubate in a humidified chamber for 30 minutes at room temperature or overnight at 4°C.
7. Rinse the slides with PBST and place the sections in a staining rack. Then immerse them in PBST buffer 4 times, 5 minutes each.
8. Secondary antibody incubation: Arrange the slides on a staining tray. Rinse off the PBST buffer and wipe the 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 recommendations for incubation temperature and time.
9. Rinse the slides with PBST buffer, then place them in a staining rack and immerse in PBST buffer 4 times, 5 minutes each.
10. Chromogenic reaction: Prepare fresh HRP-compatible chromogen solution (e.g., DAB, AEC) according to the manufacturer's instructions. Arrange the slides on a staining tray, drain the PBST, and wipe the slides. Add the chromogen solution to cover the tissue and observe the color development by eye or under a light microscope. When the desired color intensity is reached, end the reaction by rinsing the slides with distilled water, then immerse them in distilled water again, and wash the slides in running tap water for 2–5 minutes.
11. 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.

12. Dehydration: Dehydrate the tissue slides sequentially through 85% alcohol for 5 minutes, 95% alcohol for 5 minutes, and 100% alcohol for 5 minutes (twice)
13. Clearing: When the tissue slides are dried, clear the tissue in xylene or a xylene substitute for 2–5 minutes.
14. 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. Place the sections on a staining tray. Use a PAP pen to draw a hydrophobic circle around the tissue. Optionally, place the slides in distilled water after drawing to prevent drying out.
3. Block endogenous peroxidase activity: Apply the Blocking Buffer to cover the tissue sections or immerse the sections in Blocking Buffer. Incubate for 10 minutes at room temperature to quench endogenous peroxidase activity. Rinse the slides and immerse the sections in PBS buffer 5 times.
4. Primary antibody incubation:

Concentrate: Dilute the concentrated antibody at a ratio of 1:200-400 with Antibody Diluent. Apply an appropriate volume of the diluted antibody to cover the tissue section. Meanwhile, apply the negative control antibody to the tissue section according to the experiment. Incubate in a humidified chamber for 30 minutes at room temperature or overnight at 4° C.

Ready-to-Use: Apply an appropriate volume of the ready-to-use antibody to cover the tissue section. Incubate in a humidified chamber for 30 minutes at room temperature or overnight at 4°C.

5. Proceed with IHC staining following the standard protocol.

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
Liver	Nuclear, 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 Negative Control for Mouse IgG2b, kappa Primary Antibody, Cat. VN003, 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.

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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