NapsinA/p40 Cocktail Antibody

Instruction For Use

RUO

[Product Name]

NapsinA/p40 Cocktail Antibody

[Catalog No.]

SD8104

[Specification & Components]

Name	Specification
NapsinA/p40 Cocktail Antibody	1mL
	3mL
	7mL

[Materials required but not provided]

DualStacker™ Detection Kit (Dual staining I) (SD8001/SD8011)
Antigen Retrieval Solution II (SD6004)
Washing Solution Concentrate (10X) (SD6001)
Primary Antibody Diluent (SD2005)
p40/Napsin A positive control slides (lung adenocarcinoma, lung squamous cell carcinoma) ; Xylene; Ethanol (anhydrous, 95%, 85%, 70%); purified water; Neutral gum.

[Intended Use]

For Research Use Only.

This reagent is used for immunohistochemical staining based on routine staining to provide auxiliary information for physicians in diagnosis.

This reagent was used for the qualitative determination of p40 antigen and Napsin A antigen in Lung cancer sample.

[Summary and Explanation]

The Napsin A/p40 Cocktail Antibody uses antibodies to form antigen-antibody complexes with target antigens in the cells. The alkaline phosphatase (AP)-labeled anti-rabbit polymer is then bound to the p40 antibody, and the fast red staining solution is used to develop a red color, localizing to the nucleus. The horseradish peroxidase (HRP)-labeled anti-mouse polymer is bound to the Napsin A antibody, and the DAB staining solution is used to develop a brown color, localizing to the cell cytoplasm. This allows the visualization of the antigen-antibody binding sites within the cells, which can be observed under a microscope. The hematoxylin counterstaining makes the tissue structure clearer and more conducive for interpretation by the pathologist.

[Storage and Validity]

Store at 2~8℃. Avoid freezing. Valid for 12 months.

The reagent is ready-to-use . After each use, it should be put back into the refrigerator at 2 ~ 8℃.

[Recommended Instrument]

Optical Microscope (40× ~ 400×)

[Specimen Requirements]

Lung cancer tissue samples.

Lung cancer tissue samples is fresh biopsy or surgical sample tissue fixed with 10% neutral buffer formalin for 8 ~ 24h. According to the requirements of pathological technical specifications, sampling, dehydration, paraffin embedding into paraffin block. Paraffin blocks should be stored in a special, ventilated and dry paraffin block cabinet. Paraffin blocks stored at room temperature are valid for 5 years.The tissue sections with a thickness of 3 ~ 5 μm were spread on sticky slides. If the tissue slices are stored at room temperature, the detection should be completed within 7 days in order to reproduce the distribution of antigens in the tissue. If the tissue sections in cold storage (2 ~ 8℃), the detection should be completed within 3 months In order to reproduce the distribution of antigens .

[Recommended Protocol]

1. Instruments and Equipment Required for Testing
Pipette, pap pen, timer, incubation box, staining holder, coverslip, and optical microscope (40× ~ 400×), wash bottle.

2. Solution Preparation:
2.1 Washing solution:Prepared by washing solution concentrate (10x) and purified water in a ratio of 1:10.
2.2 Fast red chromogenic solution: prepared by Fast-red Part A (Substrate), Fast-red Part B (Activator) and Fast-red Part C (Buffer)=1:1:20.
Part A and Part B are mixed and reacted for three minutes, and then add fast red buffer.
2.3 DAB staining solution: prepared by 1:20 ratio of DAB Chromogen and DAB Substrate Buffer.

3. Test Temperature Condition: 18℃ ~ 25℃.

4. Test Procedures
4.1 Specimen Treatment
Deparaffinization and hydration：
Paraffin sections were placed in fresh xylene and soaked twice, 10min/ time.
After removing the excess liquid, dip the sections in anhydrous ethanol for 2 times, 5min/ time.
After removing the excess liquid, soak it in 95% ethanol for 5min, then repeat this step in 85% ethanol.
Rinse with purified water for 3 times, 3min/time.

4.2 Antigen Retrieval
Put the sample slide into the immunohistochemical antigen retrieval buffer, and repair it under high pressure for 3~5 minutes; cools down naturally to room temperature.

4.3 Add Peroxidase Block
Rinse the sample slide after antigen retrieval with purified water 3 times, 3min/time. Use a pap pen to circle the area to be tested on the slide, and wash it once with washing solution, 3min/time. To remove the washing solution, 100μL of peroxidase block was added to the tissue area to be tested and incubated at room temperature for 5min. Washed with washing solution for 3 times, 3min/time.

4.4 Add Combined Primary Antibody

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Remove the washing solution, add 100μL combined primary antibody, incubate at room temperature for 60 min. Washed with washing solution for 3 times, 3min/time.

4.5 Add Combined Secondary Antibody
Remove the washing solution, add 100μL combined secondary antibody, incubate for 30min. Washed with washing solution for 3 times, 3min/time.

4.6 Add Fast-red substrate working solution
Remove the washing solution, and add 100μL Fast-red substrate working solution, incubate for 8-10min, Washed with purified water for 3 times, 3min/time, then washed once with washing solution, 3min/time.

4.7 Chromogen
Remove the washing solution, and add 100μL fresh DAB solution, incubate for 6-8min, Washed with purified water for 3 times, 3min/time, then washed once with washing solution, 3min/time.

4.8 Counterstain
Rinsed with purified water, and added 100 μL hematoxylin staining solution, incubate for 1-3min. Then, rinsed with purified water to return blue.

4.9 Sealing
Add one drop of aqueous mounting medium to the sample area on the slide for mounting, and observe the staining results under an optical microscope. If need to be stored for a long time, the slide can be dried by dripping with aqueous mounting medium and then sealed with neutral balata.

4.10 Reading
Read under an optical microscope.

[Quality Control]

Positive Control: A positive control ensures proper tissue preparation and staining. It should be included in every test for comparison. These controls monitor the accuracy of steps and reagents but aren't for definitive diagnosis. If a positive tissue control fails to stain appropriately, deem the test sample invalid.

Blank Control: Every staining should have a blank control reagent for comparison. Use this instead of the antibody to determine non-specific staining and enhance interpretation of specific antigen site staining.

[Results Interpretation]

The staining results must be established on the basis of a successful positive control and blank control experiment. The interpretation of the staining results is: positive (+) / negative (-).

1.Positive staining result:
The cytoplasm of the target cells in the tissue section shows yellow or brown-yellow coloration, without background staining. The nuclei of the target cells show red coloration, without background staining.
2.Negative staining result:
The nuclei of the expected cells in the tissue do not show red coloration.
The cytoplasm of the expected cells in the tissue do not show yellow or brown-yellow coloration.
3.Based on the establishment of positive control and blank control experiments for the tissue:
If the test tissue section shows positive red coloration, it indicates the presence of the p40 antigen in the tissue section.
If the test tissue section shows positive yellow-brown or brown-yellow coloration, it indicates the presence of the Napsin A antigen in the tissue section.
Note: In each staining process, positive control slides and blank control reagents must be used, otherwise the results cannot be used.
4.Based on the establishment of positive control and blank control experiments for the tissue:
If the test tissue section shows negative staining, it suggests a low probability of the presence of p40 antigen and Napsin A in the tissue section.
5.If both the positive control and blank control experiments show negative results, it indicates that the reagents are invalid or there are errors in the detection operation. The detection should be repeated, and quality control should be performed on the operation process and the detection results.

[Test Limitations]

1. Expertise Required: Immunohistochemical pathology diagnosis involves multiple steps. Ensuring accuracy requires rigorous training in reagent selection, sample preparation, and staining interpretation. Standardization is best achieved through professional operators and accredited labs, minimizing variations in staining.
2. Tissue Processing Impact: The preparatory stages significantly influence staining outcomes. Mistakes in fixation, handling (e.g., freezing, thawing, washing, drying, slicing), or contamination can lead to false results or abnormal staining. Variations in fixation and embedding methods can also affect results.
3. Counter Staining Precision: Both excessive and insufficient counter staining can distort results interpretation.
4. Comprehensive Evaluation: Interpreting staining, whether positive, negative, or absent, should consider clinical history, cell morphology, and other histopathological context. This interpretation must be complemented by morphological studies, appropriate controls, and other diagnostic tests. A pathologist should integrate these findings with clinical conditions and other examinations.
5. Reagent Reactions: There's potential for unexpected reactions when using reagents on untested tissues. Due to the inherent variability in antigen expression across tumors or pathological tissues, unforeseen reactions may occur.
6. False Positives: Non-immunological protein bindings or substrate reaction products can lead to false positive results. Red blood cells and cytochrome C can also be sources of errors.

[Product Performance Specifications]

1. Compliance:
Positive control results should be positive with accurate localization and no background staining. Blank control and negative control staining should be negative.
2. Intra-batch Reproducibility:
Tissue sections from the same tissue source should exhibit consistent staining intensity and localization within the same

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batch of reagents.
3. Inter-batch Reproducibility:
Tissue sections from the same tissue source should exhibit consistent staining intensity and localization when stained with reagents from different batch numbers.

[Cautions]

1.This reagent is intended for research use only. It should not be used for any other purpose.
2.Used by professionally trained personnel only, such as qualified healthcare professionals or laboratory technicians.
3.The p40 monoclonal antibody and Napsin A monoclonal antibody used in this reagent are derived from biological resources. Their handling must comply with all relevant laws, regulations, and safety protocols.
4.When using this reagent, appropriate personal protective equipment (PPE) must be worn to avoid skin and eye contact. This includes gloves, lab coat, and eye protection as a minimum.
5.The suitability of this reagent for use with non-formalin-fixed tissue samples has not been established. Its performance has only been validated for use with formalin-fixed, paraffin-embedded tissue specimens.

[Symbols]

The following symbols may be found in this IFU or on the product labeling. Some glossary symbols may not be applicable to this product.

Symbol	Used for	Symbol	Used for
	Expiration Date		Consult instructions for use
LOT	Lot Number	RUO	For Research Use Only.
	Storage Temperature		Manufacturer
REF	Catalogue number		Date of manufacture

[Version]

Revision date: 2024-01-02
Version: 01; Change Summary: new

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