AMACR/p63/CK HMW Cocktail Antibody

Instruction For Use

IVD

[Product Name]

AMACR/p63/CK HMW Cocktail Antibody

[Catalog No.]

SD8009/ SD8019

[Specification & Components]

Name	Specification		
	100-150 tests/kit	100-200 tests/kit	300-600 tests/kit
Peroxidase Block	15ml*1	10ml*1	30ml*1
Combined Primary Antibodie	15ml*1	10ml*1	30ml*1
Combinded Secondary Antibody	15ml*1	10ml*1	30ml*1
Fast-red Part A (Substrate)	2ml*1	0.4ml*1	1.2ml*1
Fast-red Part B (Activator)	2ml*1	0.4ml*1	1.2ml*1
Fast-red Part C (Buffer)	30ml*1	10ml*1	30ml*1
DAB Chromogen (20x)	2ml*1	0.5ml*1	1.5ml*1
DAB Substrate Buffer	30ml*1	10ml*1	30ml*1
Hematoxylin	15ml*1	10ml*1	30ml*1

[Materials required but not provided]

Antigen Retrieval Solution;Wash Solution 10X Concentrate; Primary Antibody Diluent; Aqueous mounting medium; Antibody; Xylene; Ethanol (anhydrous, 95%, 85%, 70%); Purified water; Neutral gum.

[Intended Use]

For In Vitro Diagnostic Use.

In the immunohistochemical assay, the target analyte is captured by the primary antibody, and the antibody-target complex is then detected through a labeling and staining procedure.This helps convey the full process of primary antibody binding, followed by detection/visualization through labeling and staining.

[Summary and Explanation]

Using the principle of antibody antigen-specific binding, and through the redox of the secondary antibody-labeled enzyme developer color, the target for qualitative positioning. Firstly, the mouse anti-human antibody binds to the target antigen I, and the Horseradish peroxidase labeled secondary antibody binds to the mouse anti-human antibody. At the same time, rabbit anti-human antibody binds to target antigen II, and Alkaline phosphatase labeled rabbit-derived secondary antibody binds to rabbit anti-human antibody, which is shown to be red by fast red staining to qualitatively localize antigen II. Hematoxy- lin counterstaining can make the tissue structure more clear and facilitate the pathologist to interpret the results.

[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]

Preparation: Use fresh biopsy or surgical tissue samples. Fix with 10% neutral buffered formalin for 8 to 24 hours. Follow pathological technical guidelines for sampling, dehydration, and paraffin embedding to form a paraffin block.

[Recommended Protocol]

This instruction offers manual reagent operation methods for reference purposes. For on-board reagent testing methods, please refer to the machine's preset program.

1.Instruments and Equipment Required for the Test:
Pipette, immunohistochemistry marking pen, timer, incubator, staining dipper, coverslip, optical microscope, wash bottle.

2.Solution Preparation:
2.1 DAB staining solution: prepared by 1:20 ratio of DAB Chromogen and DAB Substrate Buffer.
2.2 The Fast-red substrate working solution is prepared by mixing Fast-red Part A (Substrate), Fast-red Part B (Activator), and Fast-red Part C (Buffer) in a 1:1:20 ratio.
It is critical to combine Part A and Part B first and let the mixture react for 3 minutes, then add the Part C to the pre-mixed solution.
Prepare the Fast-red substrate working solution right before use.
2.3 Wash Solution:Wash Solution 10X Concentrate and purified water 1:10 ratio.

3.Test Temperature: 18-25°C.

4.Test Procedure:
4.1 Deparaffinization and Hydration:
Place paraffin sections in fresh xylene, immerse twice for 10 minutes each time.
After removing excess liquid, place them in anhydrous ethanol, immerse twice for 5 minutes each time.
After removing excess liquid, place them in 95% ethanol, immerse for 5 minutes.
After removing excess liquid, place them in 85% ethanol, immerse for 5 minutes.
Rinse with purified water three times, 3 minutes each time.

4.2 Antigen Retrieval:
Repair by boiling in a pressure cooker for 3 minutes, then cool naturally. Soak in purified water twice for 3 minutes each time. During antigen retrieval, the volume of retrieval solution must ensure that the sections are immersed in the liquid at all times.

4.3 Add Peroxidase Block:
Rinse the sample slide after antigen retrieval with purified water

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3 times, 3min/time. Use a marking pen to circle the area to be tested on the slide, and wash it once with washing solution, 3min/time.
Remove the washing solution, and add peroxidase block (the amount of reagent depends on the size of the section and should completely cover the sectioned tissue) to the tissue area to be tested and incubated at room temperature for 5min, then rinse with wash solution three times, for 3 minutes each time.

4.4 Add Antibody:
Remove the wash solution and add primary antibody (mouse) (the amount of reagent depends on the size of the section and should completely cover the sectioned tissue). Incubate at room temperature for 60 minutes, then rinse with wash solution three times, for 3 minutes each time.

Remove the wash solution and add primary antibody (rabbit) (the amount of reagent depends on the size of the section and should completely cover the sectioned tissue). Incubate at room temperature for 60 minutes, then rinse with wash solution three times, for 3 minutes each time.

4.5 Add Combinded Secondary Antibody:
Remove the wash solution and add the Combinded Secondary Antibody (the amount of reagent depends on the size of the section and should completely cover the sectioned tissue). Incubate at room temperature for 30 minutes, then rinse with wash solution three times, for 3 minutes each time.

4.6 Add Fast-red substrate working solution:
Remove the wash solution and add Fast-red substrate working solution (the amount of reagent depends on the size of the section and should completely cover the sectioned tissue). Incubate at room temperature for 5-10 minutes, then rinse with purified water three times, for 3 minutes each time.

4.7 Add DAB Chromogen (20x):
Remove the wash solution and add DAB Chromogen (20x) (the amount of reagent depends on the size of the section and should completely cover the sectioned tissue). Incubate at room temperature for 5-10 minutes, then rinse with purified water three times, for 3 minutes each time.

4.8 Counterstain:
Add hematoxylin (the amount of reagent depends on the size of the section and should completely cover the sectioned tissue). Incubate for 3 minutes, then rinse with purified water to achieve a blue color.

4.9 Mounting:
Add a drop of aqueous mounting medium to the sample area on the slide. Let it air dry naturally or bake at 65°C. For long-term preservation, after drying, add neutral gum for mounting.

4.10 Microscopic Examination:
Examine the slides under an optical microscope.

[Results Interpretation]
Positive controls: Positive controls can be used as an indicator of correct tissue and appropriate staining counts. Each staining should include a comparison of positive controls under the same test condi- tion. Known positive tissue controls can only be used for monitoring the correct execution of the procedure and the reagent test perfor- mance, are not used to assist in the clear judgment of the patient sample. If a positive control does not show an appropriate positive stain, the results of this batch of samples should be considered invalid.

Blank control: Each staining should include blank control under the same test condition for comparison. Blank control reagent was used to stain tissue sections instead of antibodies to determine non-specific staining and to provide a better interpretation of antigen site specific staining.

[Results Interpretation]
The staining results must be based on the positive and negative control experiments:
positive: brown or red staining for the target antigen site.
Negative: no brown or no red staining.
Results Interpretation should be determined by a qualified pathologist.

[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.
7. Kit Limitations: This kit has been validated only for tissues fixed with 10% neutral buffered formalin and paraffin-embedded. It is not suitable for other specimen types or uses, such as flow cytometry.

[Product Performance Specifications]
1.Consistency
Positive control results show positive, and the positive staining is accurately located without background staining. The negative and blank controls results show negative .
2.In-batch Repeatability

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There was no significant difference in the intensity and location of cells staining from the same tissue source.
3.Inter-batch repeatability
There was no significant difference in the intensity and location of cells staining from the same tissue source with different batches of reagents respectively.

[Cautions]

1.This kit is for in vitro diagnostic use and not for other purpose.
2.Before starting experiment, please read the instruction for use carefully.
3.This reagent must be used by highly trained professionals within valid period. If leakage, contamination or deterioration is found, please do not use it.
4.If the components in the kit are mixed with other company products, it may occur abnormal staining.
5.The reagent shall be protected with appropriate measures to avoid contact with the skin and eyes.

[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	IVD	*In Vitro* diagnostic medical device
	Storage Temperature		Manufacturer
REF	Catalogue number		Date of manufacture

[Version]

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

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