MicroStacker™ AP Polymer Detection Kit (Red)

Instruction For Use

RUO

[Product Name]

MicroStacker™ AP Polymer Detection Kit (Red)

[Catalog No.]

SD8003/SD8013

[Specification & Components]

Name	Specification		
	100-150 tests/kit	100-200 tests/kit	300-600 tests/kit
Linker, Rabbit-anti-Mouse	15ml*1	10ml*1	30ml*1
Anti-Rabbit AP-Polymer	15ml*1	10ml*1	30ml*1
Fast-red Part A (Substrate)	2ml*1	0.5ml*1	1.2ml*1
Fast-red Part B (Activator)	2ml*1	0.5ml*1	1.2ml*1
Fast-red Part C (Buffer)	30ml*1	10ml*1	30ml*1
Hematoxylin	15ml*1	10ml*1	30ml*1

[Materials required but not provided]

Antigen retrieval solution for IHC (refer to IFU of primary antibody); Wash Solution 10X Concentrate; Aqueous Mounting Medium; Xylenes; Ethanol (anhydrous, 95%, 85%, 70%); purified water

[Intended Use]

For Research Use Only.

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]

MicroStacker™ AP Polymer Detection System is a highly sensitive 2-step, biotin-free, polymeric Alkaline Phosphatase secondary antibody conjugate system for the detection of mouse and rabbit primary antibody on formalin-fixed, paraffin-embedded (FFPE) tissues in an immunohistochemistry (IHC) procedure. In this 2-step amplification system, the linker reagent is applied after the primary antibody to further amplify the signal, then the enzymatic polymer is sequentially added. The innovative MicroStacker™ technology allows well-controlled layered stacking of Fab fragments of antibodies and alkaline phosphatase enzymes on a micro-polymer scaffold, which results in a compact polymeric structure that easily penetrates to all cellular compartments, and provides superior sensitivity compared to other conventional polymers with bulky dextran backbones.

[Storage and Validity]

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

[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 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.
2.2 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: Follow the instructions in the antibody reagent manual.

4.3 Mark the Targeted Sampling Area:
After the antigen retrieval, rinse the slides in purified water three times for 3 minutes each time. Use a marking pen to outline the area of the tissue to be tested on the glass slide. Wash the slides with the wash solution once for 3 minutes.

4.4 Add Antibody:
Follow the instructions in the antibody reagent manual.

4.5 Add Linker, Rabbit-anti-Mouse:
Remove the wash solution and add the Linker, Rabbit-anti-Mouse (the amount of reagent depends on the size of the section and should completely cover the sectioned tissue). Incubate at room temperature for 20 minutes, then rinse with wash solution three times, for 3 minutes each time.

4.6 Add Anti-Rabbit AP-Polymer:

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Remove the wash solution and add Anti-Rabbit AP-Polymer (the amount of reagent depends on the size of the section and should completely cover the sectioned tissue). Incubate at room temperature for 20 minutes, then rinse with wash solution three times, for 3 minutes each time.

4.7 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.

4.8 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.

[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 based on the positive and negative control experiments:
positive: the target antigen site shows red staining.
Negative: no red staining.
Results Interpretation should be determined by a qualified patholo- gist.

[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. Fase 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. 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 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 kit is for research use only and should not be used for any other purposes.
2.This reagent must be used by trained professionals within its validity period. Do not use if leakage, contamination, or deterioration is observed.
3. Store the reagent away from high temperatures and direct sunlight.
4.After use, dispose of waste according to hospital or environmental department regulations.

[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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