MicroStacker™ Goat Anti-Mouse HRP-Polymer

Instruction For Use

[Product Name]

MicroStacker™ Goat Anti-Mouse HRP-Polymer

[Specification & Components]

Name	Code	Components
MicroStacker™ Goat Anti-Mouse HRP-Polymer	CSM1001-20mL	Anti-Mouse HRP Polymer
	CSM1001-100mL	
	CSM1001-1L	

[Intended Use]

For Research Use Only

[Summary and Explaination]

MicroStacker™ Polymer Detection System is a biotin-free, polymeric horseradish peroxidase (HRP)- secondary antibody conjugate system for the detection of mouse primary antibody on formalin-fixed, paraffin-embedded (FFPE) human tissue in an immunohistochemistry (IHC) procedure. The innovative MicroStacker™ technology allows well-controlled layered stacking of antibodies and peroxidase enzymes on a micro-polymer scaffold, which avoids the occasional blocking of the antibody binding site during the labeling process. This technique results in a compact polymeric structure that easily penetrates to all cellular compartments, which provides superior sensitivity compared to other conventional HRP polymers with bulky dextran backbones. Plus, the system utilizes Fab' fragments of IgG secondary antibody instead of the whole IgG, which avoids the background caused by non-specific binding of whole IgG to endogenous Fc receptors.

[Storage and validity]

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

[Specimen Requirements]

Tissue Preparation: Fix with 10% neutral buffered formalin for 8 to 24 hours. Follow pathological technical guidelines for sampling, dehydration, and paraffin embed- ding to form a paraffin block.

Storage: Store paraffin blocks in a dedicated, well-ventilated, dry cabinet. Blocks remain viable for 5 years at room temperature.

Slide Preparation: Spread tissue sections, 3-5μm thick, onto adhesive slides. Absorb excess moisture with absorbent paper and gently pat on the slide rack. Bake the sections in an oven at 60℃ (±5℃) for 30 to 60 minutes or let them sit overnight at 37℃. Detection Timelines: For tissue slices at room temperature, complete detection within 7 days to ensure antigen distribution integrity. For slices stored at 2-8 ℃, complete detection within 3 months.

[Instruments & Equipment]

Pipette, immunohistochemical pap-pen, timer, drying box, incubation box, staining holder, coverslip, optical microscope, wash bottle.

[Test Temperature]

Maintain between 18-25℃.

[Recommended Protocol]

1.Deparaffination: Immerse slides in xylene. Hydrate using a graded series of alcohols ending in water.

2.Peroxidase Block: Treat for 5 minutes with Peroxidase Blocking Reagents. Rinse twice using wash buffer for 5 minutes each.

3.Antigen Retrieval: Refer to the primary antibody data sheet for the recommended protocol.

4.Primary Antibody: Apply 2 drops (~100μl) or as needed of the primary antibody to cover the specimen. Incubate 60 minutes at room temperature (RT). Rinse twice using wash buffer for 5 minutes each.

5.Detection Polymer: Apply 2 drops (~100μl) or required amount of anti-Mouse HRP-Polymer. Incubate for 30 minutes at RT. Rinse twice with wash buffer for 5 minutes each.

6.DAB Chromogen: Prepare ready-to-use DAB substrate solution. Typically, 100μl is sufficient for one slide. Incubate slides for 5 minutes at RT, then rinse with deionized water.

7. Counterstain: Use hematoxylin and rinse with deionized water.

8.Dehydration & Sealing: Successively immerse slides in 70%, 85%, and 95% ethanol for 2 minutes each. Soak in anhydrous ethanol twice for 2 minutes each time. Transparentize with xylene twice, 2 minutes each time. Seal with neutral balata and a coverslip.

9.Observation: Examine 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 brown staining. Negative: no brown staining.

Results Interpretation should be determined by a qualified pathologist.

[Test Limitations]

1.Expertise Required: Immunohistochemical experiment 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 substrateT 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.

[Cautions]

1.For Research Use Only.
2.Preparation: Carefully review the instruction for use prior to experimentation.
3.Professional Use: Only trained professionals should use the reagent within its validity period. Do not use if leakage, contamination, or deterioration is observed.
4.Compatibility: Mixing components with products from other manufacturers may result in abnormal staining.
5.Safety Precautions: Avoid skin, eye contact, or inhalation. In case of contact, promptly rinse the affected area with ample water.
6.Control Slides: Every staining must include both a positive contrast slide and a blank control. Without these controls, results are inadmissible.
7.Procedure Adherence: Incorrect antigen retrieval, incubation duration,temperature, or other methods can skew results.
8.Sample Freshness: Samples stored at room temperature should be processed within 7 days to prevent antigen degeneration and potential false negatives.
9.Control Indications: If a positive control fails to stain correctly, this suggests procedural error, rendering the sample batch results invalid.
10. DAB Safety: Given DAB's potential mutagenicity, ensure safety precautions during use. Waste disposal should adhere to relevant legal requirements.
11. Kit Limitations: The efficacy of this kit on non-formalin fixed tissues remains unverified.

[Basic Information]
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