MicroStacker™ Goat Anti-Mouse AP-Polymer

Instruction For Use

[Product Name]

MicroStacker™ Goat Anti-Mouse AP-Polymer

[Specification & Components]

Name	Code	Components
MicroStacker™ Goat Anti-Mouse AP-Polymer	CSM1011-2mL	Anti-Mouse AP Polymer
	CSM1011-20mL	
	CSM1011-100mL	
	CSM1011-1000mL	

[Intended Use]

For Research Use Only

[Summary and Explanation]

MicroStacker™ Goat Anti-Mouse AP-Polymer is a biotin-free, polymeric Alkaline Phosphatase (AP)- 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 alkaline phosphatase 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. 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. 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.

2. Antigen Retrieval: Follow the instructions in the antibody reagent manual.

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 pap-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. 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 AP-Polymer. Incubate for 30 minutes at RT. Rinse twice with wash buffer for 5 minutes each.

6. Incubate with Fast-red Substrate Working Solution:

Apply 2 drops (~100ul) or required amount of Fast-red substrate working solution. Incubate at room temperature for 6-8 minutes, then rinse with purified water three times, for 3 minutes each time.

7. Counterstain:

Add hematoxylin (the amount of reagent depends on the size of the section and should completely cover the sectioned tissue). Incubate for 1-3 minutes, then rinse with purified water to achieve a blue color.

8. Mounting:

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

9. Microscopic Examination.

[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 is observed.

[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.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.

5. 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.This reagent kit is for research use only and should not be used for any other purposes.

3.Preparation: Carefully review the instructions for use prior to experimentation.

4.Only trained professionals should use the reagent within its validity period. Do not use if leakage, contamination, or deterioration is observed.

5.Avoid contact with skin and eyes, or inhalation. Avoid contact with skin and eyes, or inhalation, promptly rinse the affected area with ample water.

6.Every staining must include both a positive contrast slide and a blank control. Without these controls, results are inadmissible.

7.Variations in antigen retrieval, incubation times, temperature conditions, or the application of other methods may lead to erroneous results.

[Basic Information]
Henan Celnovte Biotechnology, Ltd.

Address: N0.1 Cuizhu Street, Bldg. 109, Hi-tech District, Zhengzhou, Henan, China. 450000

Tel: +86(371)-56596916