RUO (Research-use-Only)

MicroStacker™ Goat Anti-Mouse HRP-Polymer Detection Kit Instruction For Use

[Product Name]

MicroStacker™ Goat Anti-Mouse HRP-Polymer Detection Kit

[Packing Specification]

200 test/kit, 300 test/kit, 1000 test/kit

[Intended Use]

In the immunohistochemical reaction, the target is bound to the primary antigen antibody and labeled by staining.

[Test Principle]

The MicroStacker™ Goat Anti-Mouse HRP-Polymer Detection Kitt uses principle that the antibodies and target antigens on the slice form antigen-antibody complexes, then enzyme labeled goat anti-mouse polymer combine with the antibodies in the antigen-antibody complexes, finally horseradish peroxidase catalytic diamino benzidine (DAB) form brown precipitate in antigen, and with the aid of a microscope observe its color changes, thus to determine the organization, the structure of the cell in antigen-antibody combining site. Hematoxylin counterstaining can make the tissue structure more clear and facilitate the pathologist to interpret the results.

[Main Components]

It is mainly composed of endogenous peroxidase blocking reagent, enzyme-labeled goat anti-mouse polymer, DAB substrate (20×), substrate buffer and hematoxylin.

[Storage and validity]

Store at 2~8℃ avoid freezing, valid for 12 months.

[Recommended Instrument]

Optical microscope (40× ~ 400×)

[Specimen Requirements]

Fresh biopsy or surgical sample tissue fixed with 10% neutral buffered formalin for 8 ~ 24h. According to the requirements of pathological technical specifications, sampling, dehydration, paraffin embedding and make into paraffin block. The paraffin blocks shall be stored in a special, ventilated, and dry cabinet. Paraffin blocks stored at room temperature valid for 5 years.

The tissue sections with a thickness of 3 ~ 5 m were spread on adherent slides. Remove water in the tissue sections by gently patted on the slide rack and absorbing with hygroscopic paper. The tissue sections were then placed in a drying oven at 60℃ (±5℃) for 30 ~ 60min or placed overnight at 37℃.

If the tissue slices are stored at room temperature, the detection should be completed within 7 days to reproduce the distribution of antigens in the tissue. In cold storage (2 ~ 8℃), the detection should be completed within 3 months to reproduce the distribution of antigens.

[Test Method]

1. instruments and equipment

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

2. Solution preparation

DAB chromogenic solution: Add 1mL substrate buffer and 50uL DAB substrate (20×) to the centrifuge tube. Store avoid light.

3. Test temperature required: 18~25℃

4. Experimental procedures

4.1 Deparaffinization and hydration

Place paraffin sections in fresh xylene and soaked twice, 10min/ time.

After removing the excess liquid, placed it in anhydrous ethanol and soaked for 2 times, 5min/ time. Remove excess liquid, soak in 95% ethanol for 5min, repeat the procedure in 85%. Rinse with tap water for 3 times, 3min/ time.

4.2 Antigen retrieval: Refer to the antibody reagent manual.

4.3 Add endogenous peroxidase blocking reagent

Rinse the sections after antigen retrieval with tap water for 3 times, 3min/ time. The test tissue area on the slide was defined with an oil pen. Rinsed with the TBS washing solution once, 3min/ time.

Remove the TBS washing solution, added 100 μL of endogenous peroxidase blocking reagent to the defined area and incubate at room temperature for 5min.

Rinse with TBS washing solution for 3 times, 3min/ time.

4.4 Add antibody

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

4.5 Add enzyme-labeled goat anti-mouse polymer

Remove the TBS washing solution, add 100 μL enzyme-labeled goat anti-mouse polymer, and incubate at room temperature for 30min.Rinse with TBS washing solution for 3 times, 3min/ time.

4.6 chromogen

Remove the TBS washing solution was and add 100 μL fresh DAB chromogenic solution, then incubate at room temperature for 5min.

4.7 counterstain

Rinse with tap water and incubate with 100μL hematoxylin for 3min. The rinse with running tap water to return blue.

4.8 Dehydrate, transparentize and seal

Immerse in 85% ethanol ,95% ethanol for 2min, respectively. Soak in anhydrous ethanol for 2 times,2min/ time.

Xylene was used to make the sample transparent by soaking the slide in twice, 2min/ time. Use neutral balata and coverslip to seal slip.

4.9 Read by Optical microscope

5. Quality control

5.1 Positive control

A positive control can be used as an indicator of proper tissue preparation and appropriate staining techniques.

Each dye should include a positive under the same test condition to contrast.

Known positive tissue controls can only be used to monitor the correct execution of steps and reagent tests, are not used to assist in describing a definitive diagnosis of the patient sample.

If a positive tissue control does not show an appropriate positive stain, the results of this test sample shall be considered invalid.

5.2 Blank control

Each staining should include a blank control reagent for comparison under the same test conditions.

The blank control reagent was used instead of the antibody to stain the tissue sections to determine the non-specific staining and provide a better interpretation of the specific staining of antigen sites.

[Reference Range]

The kit is a staining reagent, has no reference range.

[Results Interpretation]

The staining results must be based on the positive and blank control experiments:

positive: the target antigen site shows brown staining.

Negative: No target antigen shows no brown staining.

Results Interpretation should be determined by a qualified pathologist.

[Test Limitations]

1. Immunohistochemical pathology diagnosis is a multi-step diagnostic process. Reagent selection, sample fixation, processing, section preparation and interpretation of staining results must undergo rigorous professional training; Professional operators and accredited laboratories will contribute to

the standardization of the experimental testing process, thus reducing staining deviations due to external factors.

2. The processing of tissues before staining directly affects the dyeing effect. Improper fixation, freezing, melting, washing, drying, slicing, or contamination with other tissues or liquids can result in false positives, inaccurate antibody location, or false negative results. Different fixation and embedding methods or irregular within the tissue may also result in abnormal staining results.

3. Excessive or insufficient counter staining will affect the interpretation of the results.

4. The clinical explanation for any positive or negative staining or staining absence must be evaluated based on clinical history, cellular morphology, and other histopathological background. Any clinical explanation for staining or its absence must be supplemented by morphological studies and correct control and other diagnostic tests. The test results and diagnostic value should also be analyzed and evaluated by the pathologist combining with clinical condition and other examination results.

5. Reagents may react unexpectedly on previously unverified tissues. Because of the biological variability of antigen expression in tumor or other pathological tissues, cannot eliminate the possibility of unexpected responses caused by stimulation in the tested tissues.

6. False positive results may be due to non-immunological binding of proteins or substrate reaction products or may be due to red blood cells and cytochrome C.

7. The kit has only verified the paraffin-embedded tissues fixed with 10% neutral buffered formalin and cannot be used for other specimen types or flow cytometry and other purposes.

[Product Performance]

1.pH: the pH value of enzyme-labeled goat anti-mouse polymer is 7.5~7.7, the pH value of substrate buffer is 7.5~7.7.

2. In-batch repeatability: CD3 antibody reagent (immunohistochemical), Cytokeratin 7(immunohistochemical) and Ki-67 antibody reagent (immunohistochemical) were detected tissue slices of tonsil, lung and breast with the same batch number product, and there was no significant difference in the intensity and location of tissue slice staining.

3. Inter-batch repeatability: CD3 antibody reagent (immunohistochemical), Cytokeratin 7(immunohistochemical) and Ki-67 antibody reagent (immunohistochemical) were detected tissue slices of tonsil, lung and breast with three different batches of products, and there was no significant difference in the intensity and location of tissue slice staining.

[Cautions]

1. The kit is an in vitro diagnostic reagent and should not be used for other purposes.

2. Before the experiment, read the instruction for use carefully.

3. The reagent must be used within the validity period by strictly trained professionals. If leakage, contamination, or deterioration are found, do not use it.

4. Abnormal staining may occur if the components in the kit are mix used with products from other companies.

5. Proper protective measures should be taken to avoiding contact with the skin and eyes and should not be inhaled into the mouth. If the reagents contact with the skin, mucosa and other parts of the body, a large amount of water should be used in time to wash the reagents.

6. During each staining, positive contrast slide and blank control must be used, otherwise the results cannot be used.

7. Improper antigen retrieval, incubation time, temperature conditions, or other application methods may lead to incorrect results.

8. When stored at room temperature, the samples should be stained within 7 days. Otherwise, the degeneration of the antigen in the tissue can produce false negative results.

9. If a positive control does not show an appropriate positive staining, indicates an operational error and the results of this batch of samples shall be invalid.

10.DAB has a potential mutagenic effect, Special attention should be paid to safety precautions during use. The storage and detoxication of waste liquid after use should also comply with relevant laws and regulations

11. The application to non-formalin fixed tissues of this kit has not been confirmed.

[Symbols]

Symbol	Used for	Symbol	Used for
	Use-by date		Consult instructions for use

LOT	Batch code	IVD	In vitro diagnostic medical device
	Temperature limit		Manufacturer
	Avoid over exposure to the sun		Date of manufacture

[Basic Information]

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