

## **CD99 Antibody Reagent (Immunohistochemical)**

### **Instruction For Use**

#### **[Product Name]**

CD99 Antibody Reagent (Immunohistochemical)

#### **[Specification]**

1mL/ Vial, 3mL/ Vial, 6mL/ Vial.

#### **[Intended Use]**

This reagent is used for immunohistochemical staining based on routine staining to provide auxiliary information for physicians in diagnosis.

This reagent was used for the qualitative determination of CD99 antigen in 10% neutral buffered formalin fixed paraffin-embedded tissue section cells. The test results are for clinical reference only. Any interpretation of positive or negative results should be made by the pathologist in combination with path morphology, clinical manifestations and other test methods, and should not be used as a separate diagnostic indicator.

#### **[Principle of Detection]**

Based on the principle of specific binding by the complementary function and affinity of immunological antigens and antibodies in molecular biology, and through the Redox Reaction, the enzyme labeled antibody catalyzes the color development of chromogenic agents to display the antigens in tissue cells, and to locate the antigens. First, the mouse anti-human CD99 immunohistochemical monoclonal antibody coupled with CD99 antigen on the tissue. Secondly, the enzyme-labeled sheep anti-mouse/rabbit IgG polymer recognizes CD99 antibodies that have been attached. Thirdly, adding color substrates, horseradish peroxidase on the polymer can catalyze the decomposition of  $H_2O_2$  in the chromogenic solution of DAB, thus benzidine is oxidized into Biphenyl imide, and yellow or brownish yellow staining appears on the antigen sites in the tissue sections. Finally, the samples were re-stained and sealed. The presence and condition of CD99 on the tissue sections were inferred by observing the coloration situation through the microscope. As a quality control, paraffin-embedded thymoma tissues fixed with 10% neutral buffered formalin were used as positive controls. The cell stain is located in the cell membrane.

#### **[Main Components]**

Mouse anti-human CD99 immunohistochemical monoclonal antibody (source: cell culture supernatant).

Components not included in the reagent but necessary for the test:

Micro Stacker™ Polymer Staining Kit; Antigen retrieval buffer II; Washing solution: diluted from concentrated washing solution (10x);

Blank control reagent: antibody dilution.

Other reagents not provided:

Positive contrast slide(thymoma); Xylene; Ethanol (anhydrous, 95%, 85%, 70%); Purified water; Neutral balata.

[Storage Conditions and Expiry Date]

Stored at 2 ~ 8°C, valid for 12 months.

The reagent is ready-to-use . After each use, it should be put back into the refrigerator at 2 ~ 8°C immediately and finished within six months.

### **【Applicable Instrument】**

Optical microscope (40× ~ 400×)

### **[Sample Requirements]**

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

The tissue sections with a thickness of 3 ~ 5 μm were spread on sticky slides. Remove the excess water in the tissue sections by gently patting on the slide stand and absorbing with hygroscopic paper. The sections were then placed in a drying oven at 60°C (±5°C) for 30 ~ 60min or placed overnight at 37°C.

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

### **[Test method]**

#### 1. Instruments and equipment required for testing

Pipette, induction cooker , timer, incubation box, staining holder, stainless steel pressure cooker and optical microscope (40× ~ 400×).

#### 2. Solution preparation:

2.1 Washing solution: Diluted from concentrated washing solution (10×).

2.2 DAB staining solution: prepared by 1:20 ratio of DAB substrate and DAB buffer solution.

3. Test temperature condition: 18°C ~ 25°C.

#### 4. Test procedures

##### 4.1 Manual test procedures

#### 4.1.1 Deparaffinization and hydration

Paraffin sections were placed in fresh xylene and soaked twice, 10min/ time.

After removing the excess liquid, dip the sections in anhydrous ethanol for 2 times, 5min/ time.

After removing the excess liquid, soak it in 95% ethanol for 5min, then repeat this step in 85% ethanol and 70% ethanol respectively and in sequence.

Rinse with purified water for 5min.

#### 4.1.2 Antigen retrieval

Use induction cooker to heat antigen retrieval buffer II in the pressure cooker to boiling.

The deparaffined and hydrated slices were placed on a heat-resisting staining holder and put into the pressure cooker.

Cover the pot and continue heating until spray steam. After 2.5min, remove the pressure cooker from heat source and cools down naturally for 5min.

Continually cool down with tap water, remove the valve and open the cover. Remove the slice out from liquid in the pot after it cools down to room temperature naturally.

Soak in purified water was twice, 3min/ time.

Soak in the washing solution once, 3min/ time.

Note: During antigen retrieval, the amount of retrieval solution must ensure that the slices are always immersed in the liquid.

#### 4.1.3 Add endogenous peroxidase blocking solution

To remove the washing solution, 100 $\mu$ L of endogenous peroxidase sealant was added to the tissue area to be tested and incubated at room temperature for 5min. Then soak the washing solution for 2 times, 5min/ time.

#### 4.1.4 Add CD99 antibody reagent or blank control reagent

Remove the washing solution, add 100 $\mu$ L CD99 antibody reagent or blank control reagent, incubate at room temperature for 60min, soak in the washing solution twice, 5min/ time.

#### 4.1.5 Add enzyme-labeled goat anti-mouse/rabbit polymer

Remove the washing solution, add 100 $\mu$ L enzyme-labeled goat anti-mouse/rabbit polymer, incubate for 30min, soak in the washing solution twice, 5min/ time.

#### 4.1.6 Chromogen

Remove the washing solution, and add 100 $\mu$ L fresh DAB solution, incubate for 5-8min, and the staining results were generally observed under an optical microscope within 10min.

#### 4.1.7 Counterstain

Rinsed with purified water, and added 100  $\mu$ L hematoxylin staining solution, incubate for 3 ~ 5min. Then, rinsed with purified water to return blue.

Note: depending on the strength of the active hematoxylin solution and the incubation time, the staining results may in a reaction of light blue to dark blue in the nucleus, while overstain or understain may affect the judgment of correct results.

#### 4.1.8 Dehydration, transparency, and sealing

70% ethanol immersion for 2min; Immerse in 85% ethanol for 2min; Soak in 95% ethanol for 2min, in sequence.

Then dip into the anhydrous ethanol twice, 2min/ time.

Finally, transparentize with xylene, sealed with neutral balata and coverslip.

4.2 Instrument test procedures: Please refer to the manual of automatic immunohistochemical staining system for specific test methods.

### 5. Quality control

5.1 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 condition. Known positive tissue controls can only be used for monitoring the correct execution of the procedure and the reagent test performance, 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.

5.2 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. The incubation time of the blank control must be consistent with the antibody.

#### **[Positive judgment value]**

1. The immunohistochemical staining results must be based on the establishment of positive control of the tissue and the blank control experiment. The interpretation of the staining results: positive (+)/negative (-);

1.1 Positive staining results show yellow or brownish on the cell membrane of specific cells in the tissue section with no background coloring.

1.2 Negative staining result show no yellow or brownish staining in the tissue cells.

2. Based on the positive and blank control experiments, the presence of positive staining in the tested section indicates the presence of CD99 antigen in the tissue sections.

3. Based on the positive and blank control experiments, negative staining in the tested section indicates that there is little or low possibility of CD99 antigen in the tissue sections.

4. If both the positive control and the blank control experiments show negative results, indicating reagent failure or experimental operation error, the experiment should be retried. Quality control should be used for operation process and test results.

### **[Results Interpretation]**

1. Incorrect results may result from antigen retrieval, incubation time, temperature conditions, or application of other methods.
2. During each staining, positive contrast slides and blank control must be used, otherwise the results cannot be used.
3. When stored at room temperature, the samples should be dyed within 7 days. Otherwise, due to long time storage, the degeneration of the antigen in the tissue can produce false negative results.
4. If the positive control does not show the appropriate positive staining, it indicates an operational error and the results of this batch of samples should be invalid.

### **[Limitations of detection Methods]**

1. Immunohistochemical detection is a diagnostic process that needs to be completed through multiple detection steps. Strict professional training is required in the selection of reagents, sampling, fixation, processing, preparation of sections and interpretation of staining results.
2. Improper tissue processing before staining directly affects the staining effect, resulting in false positive, inaccurate antibody positioning or false negative results. The inconsistencies may be due to different fixation and embedding methods or inherent differences in tissue samples.
3. Excessive or insufficient counter-staining will affect the correct interpretation of the results.
4. The clinical explanation for any positive or negative staining or staining absence must be evaluated on the basis of 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 unconfirmed tissues. Because of the biological variability of antigen expression in tumor or other pathological tissues, cannot completely 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.

### **[Product Performance]**

1. Consistency

Performed CD99 positive control and negative control according to the operation method in the product instructions, and the cell membrane were accurately located. The positive results showed yellow or brown staining, with no background coloring. The negative and blank controls of CD99 showed no coloring.

2. In-batch repeatability

Three CD99 positive sections from the same tissue source were taken and immunohistochemical test was conducted with reagent of same batch. The positive results showed that yellow or brown-yellow staining on the cell membrane, and the location of positive staining should be accurate without background staining. There was no significant difference in staining intensity and location of tissue slices.

### 3. Inter-batch repeatability

Three CD99 positive slices from the same tissue source were taken, and immunohistochemical tests were conducted with three different batches of reagents respectively. The test results showed that there was no significant difference in staining intensity and location of tissue slices from the same tissue source with different batches of reagents.

### [Cautions]

1. This reagent belongs to in vitro diagnostic reagent and is not used for other purpose.
2. This antibody must be used by highly trained professionals.
3. Mouse anti-human CD99 immunohistochemical monoclonal antibody is derived from biological resources, and its treatment should comply with relevant laws and regulations.
4. The reagent shall be protected with appropriate measures to avoid contact with the skin and eyes.
5. Whether this reagent is applied to non-formalin fixed tissues has not been confirmed.

### [Symbols]

Symbol	Used for	Symbol	Used for
	Use-by date		Consult instructions for use
	Batch code		In vitro diagnostic medical device
	Temperature limit		Manufacturer
	Avoid overexposure to the sun		Date of manufacture

**[Basic Information]**



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