Smooth Muscle Myosin antibody reagent (immunohistochemistry) instructions

[product name]

General name: Smooth Muscle Myosin Antibody reagent (immunohistochemistry)

[packing specifications]

0. 1mL / bottle, 0.2mL / bottle, 0.5mL / bottle, 1mL / bottle, 2mL / bottle, 3mL / bottle, 6mL / bottle, 7mL / bottle, 10mL / bottle.

[Expected Use]

Immunohistochemical staining is performed on the basis of routine staining (e. g., HE staining) to provide auxiliary information for diagnosis.

This reagent was used for the qualitative detection of cellular Smooth Muscle Myosin antigens in 10% neutral-buffered formalin-fixed, paraffin-embedded tissue sections. The test results are for clinical reference only, and the interpretation of any positive or negative results should be performed by the pathologist combining pathomorphology, clinical manifestations and other testing methods, not as separate diagnostic indicators.

[Detection principle]

The principle of specific binding of the molecular biological function and affinity of each other is used, and the redox reaction enables the enzyme to display the antigen in tissue cells, and its localization and qualitative. First, the monoclonal antibody linking myosin heavy chain (smooth muscle) and Smooth Muscle Myosin antigen on tissue; second, the enzyme sheep anti-mouse / rabbit IgG polymer recognizes the attached Smooth Muscle Myosin antibody, and third, the addition of chromogenic substrate on which horseradish peroxidase can catalyze H in the DAB chromosolution$_2O_2$To ze biphenidine to biphenimine, resulting in yellow or brownish coloring on the antigen site in the tissue section; finally restain and seal the sample. The presence and presence of Smooth Muscle Myosin on the tissue sections were inferred by microscopy. For quality control, the use of 10% neutral-buffered formalin-fixed paraffin-embedded human breast cancer tissue sections is recommended as a positive control. Cell staining was localized to the cytoplasm.

[Main components]

Myosin heavy chain (smooth muscle) monoclonal antibody (antibody source: cell culture supernatant) (species genus: mouse; clone number: SMMS1). Fractions not included in the reagent but are necessary for this test:

Immunochromogenic reagent (Sanot Product Record No.: 20180257; containing endogenous peroxidase seal, anti-mouse / rabbit polymer, DAB substrate (20), substrate buffer and hematoxylin);

Antigen repair buffer (Senot product record No.: YZZ 20140006);

Cleaning liquid: use the concentrated cleaning liquid (10x) (Senot product record number: Yuzhou Yaobei No. 20140002) dilution;

Blank control reagent: antibody diluent (Sanot Product Record No.: YZZ 20180260).

Other reagents not provided:

Smooth Muscle M y osin positive photo (breast cancer); xylene; ethanol (anhydrous, 95%, 85%, 70%); purified water; neutral gum.

[Storage conditions and validity]

Temperature: 2-8℃, valid for 12 months.

Transportation conditions: the transportation temperature is not higher than room temperature, and the transportation time does not exceed one week.

After opening, take the principle of take and use. After each use, it should be put back into 2~8℃ refrigerator and use within six months.

[Applicable Instruments]

Optical Microscopy (40 ~400)

[Sample Requirements]

Tissue from fresh biopsy or surgical samples, 10% neutral buffered formalin fixed for 8 to 24 h, was extracted, dehydrated, paraffin embedded and made into wax blocks according to the pathology specifications. Wax blocks shall be stored in ventilated, dry, special wax block cabinets. The wax blocks were kept at room temperature for 5 years.

Spread a thickness of 3 to 5 m on the adhesive slide, tap the slide holder and the hygroscopic paper to absorb the excess water droplets in the tissue section, then put it in a dry tank of 60℃ (± 5℃) for 30 to 60 min or at 37℃ overnight, removed from the incubator and cooled at room temperature.

The test should be done within 7 days to keep the tissue sections in cold storage (2~8℃) and within 3 months to well reproduce the distribution of the antigen in the tissue.

[method of calibration]

1. Instruments and equipment required for testing:
   Pipetter, induction cooker, timer, incubation box, staining holder, stainless steel pressure cooker, and light microscope (40 ~400).
2. Solution preparation:

2.1 Cleaning liquid: dilute with concentrated cleaning liquid (10).
2.2 DAB staining solution: DAB substrate and DAB buffer 1 ： 20 ratio preparation.
3. Test temperature
condition: 18℃
~25℃.
4. Test steps:
4.1 Manual test steps:
4.1.1, Dewaxing and hydration
Paraffin sections were placed in fresh xylene and soaked twice, 10min / time;
After removing excess liquid, put anhydrous ethanol, soak it twice, 5min / time;
After removing the excess liquid, put in 95% ethanol and soak for 5min;
After removing the excess liquid, put in 85% ethanol and soak for 5min;
After removing the excess liquid, put in 70% ethanol and soak for 5min;
Wash lush purified water for 5min;
4.1.2 Antigen repair
Heat the pressure cooker on the induction cooker to boil;
Dewaxed and hydrated sections were placed on a high temperature resistant staining holder and placed in a pressure cooker;
Cover the pot and continue to heat until 2.5min, the pressure cooker leaves the heat source and cool naturally for 5min; cool with tap water, remove the valve cap and remove the liquid from the liquid to room temperature;
Soak the purified water twice, 3min / time;
Soak in the cleaning solution once, 3mi n / time.
Note: During antigen repair, the amount of repair fluid must ensure that the slices are always immersed in the liquid.
4.1.3 Add an endogenous peroxidase sealer
The cleaning solution was removed, and 100 L of endogenous peroxidase sealant was added to the tissue area to be tested and incubated with the solution at room temperature for 5min.Then the cleaning solution was soaked twice, 5min / time.
4.1.4 Add Smooth Muscle My osin antibody reagent or blank control reagent
The cleaning solution was removed, and 100 L of Smooth Muscle Myosin antibody reagent or blank control reagent was added, incubated at room temperature for 60min, and the cleaning solution was soaked twice for 5min / time.
.1.54 A HRP-labeled sheep against mouse / rabbit polymer was added
The cleaning solution was removed and dripping with 100 L labeled sheep anti-mouse / rabbit polymer, incubated for 30min and soaked the cleaning solution twice for 5min / time. 4.1.6 Color development
The cleaning solution was removed, and 100 L of freshly prepared DAB color development solution was added and incubated for 5 to 8 min. The staining results observed by light microscopy generally did not exceed 10min.
4.1.7 Restain
The purified water was rinsed, incubated with 100 L of hematoxylin staining solution for 3 to 5 min, and rinsed to blue.
Note: According to the intensity of the acting hematoxylin staining solution and the length of incubation time, comparing the staining results leads to light blue to dark blue nucleus, and overdye or insufficient staining may endanger the correct results.
4.1.8 Dehydration, transparency and sealing
Soak in 70% ethanol for 2min;
Soak in 85% ethanol for 2min;
Soak in 95% ethanol for 2min after removal;
Then soak in anhydrous ethanol 2 times, 2min / time;
Last xene transparent, neutral gum and coverslip slips.
4.2 Instrument test procedures
Refer to the operation manual of the fully automatic IHC stain instrument for the specific test methods.
5. Quality control
5.1 Positive control
Positive controls can be used as an indication of proper tissue preparation and appropriate staining techniques.Each staining should include positives from the same test condition for photo comparison.Known positive tissue controls can only be used to monitor the correct execution of steps and testing of reagents and are not used to help describe the disease

Definitive diagnosis of a human sample. If positive tissue controls do not show appropriate positive staining, the results of this batch of experimental test samples should be considered invalid.

5.2 Blank control

Each staining included a blank control reagent under the same test condition for comparison. Blank control reagents were used to stain tissue sections instead of antibodies to judge non-specific staining and to provide a better explanation for antigen-site-specific staining.

[Positive judgment value]

1. The staining results must be based on the positive tissue control and blank control experiments, and the interpretation of the staining results is: positive (+) / negative (-). 1.1 Positive staining results indicate the appearance of yellow or brown yellow on the cytoplasm of specific cells in tissue sections with no background staining.

1.2 Negative staining results showed that no yellow or brown coloration appeared in the expected cells within the tissue.

2. On the basis of the tissue positive control and blank control experiments, the positive staining in the examined tissue piece indicates the presence of Smooth Muscle Myosin antigen on the tissue section.

3. On the basis of the tissue positive control and blank control experiments, the negative staining in the examined tissue piece indicates the low possibility of Smooth Mu scle Myosin antigen on the tissue section.

4. If the tissue positive control and blank control experiments are negative, indicating that the reagent fails or the detection operation error, re-test shall be conducted, and the operation process and test results shall be quality controlled.

[Interpretation of the test results]

1. Antigen repair, incubation time, temperature conditions, or application of other methods may lead to false results.

2. Positive photo pairs and blank control reagents must be used during each staining process, otherwise the results must not be used.

3. Samples should be stored at room temperature and should be stained within 7 days of storage. Otherwise, it will produce false negative results of antigen degeneration in the tissue because of a long time.

4. If the positive tissue control cannot show the appropriate positive staining, then it indicates an operational error, and the results of this batch of test samples should be invalid.

[Limitations of the detection method]

1. Immunohistochemistry pathological diagnosis is a multi-step diagnostic process, reagent selection, sample fixation, processing, section preparation and interpretation of staining results must have strict professional training;

2. Processing of the tissue before staining directly affects the staining effect. Improper fixation, freezing, melting, cleaning, drying, slicing, or contamination with other tissue or liquid can all cause false positives, inaccurate antibody positioning, or false negative results.

3. Different fixation and embedding methods or irregularities within the tissue may also cause abnormal staining results. At the same time, excessive or insufficient restaining will affect the correct interpretation of the results;

4 The clinical interpretation of any absence of positive or positive staining must be evaluated with reference to their clinical history, cytomorphology, and other histopathological backgrounds. The clinical interpretation of any staining or its absence must be supplemented by morphological studies and correct control and other diagnostic tests. Test results and diagnostic value should also be comprehensively analyzed and judged by the pathologist in combination with clinical and other examination results;

5 Reagents may experience unexpected reactions on previously untested tissues. Given the biological variability of antigen expression in the tumor or other pathological tissue, the stimulation cannot completely eliminate the possibility of an unexpected response in the tested tissue;

6 False positive results may result from non-immunological binding of protein or substrate reaction products, or from red blood cells and cytochrome C.

[Product performance index]

1. Compliance

Smooth Muscle Myosin positive photos and negative photos were taken for immunohistochemical testing according to the product instructions, accurate cytoplasm, positive showed yellow or brown yellow, no background coloring, Smooth Muscle Myosin negative negative for photos and blank control, no coloring.

2. Within-batch repeatability

Smooth Muscle Myosin positive sections from the same tissue source were taken and tested in parallel three times with the reagent of the same batch number. The test results were that the cytoplasm of the tissue cells appeared yellow or brown and was positive. The positioning of positive coloring should be accurate without background coloring.

3. Reproducibility between batches

Smooth Muscle Myosin positive sections from the same tissue source were taken and tested three times with three different batches. There was no obvious difference in the intensity and localization of staining of tissue slices from the same tissue source.

[matters need attention]

1. This reagent is an in vitro diagnostic reagent and has no other purpose;
2. The antibody must be used by strictly trained professionals;

3. Myosin heavy chain (smooth muscle) monoclonal antibody comes from biological resources, and its treatment should meet the requirements of phase laws and regulations;

4. Appropriate protective measures shall be taken to avoid reagent contact with the skin and eyes;

5 The application of this reagent to non-formalin-fixed tissue has not been confirmed.

[reference documentation]

1. Wu Bingquan, Liu Yanqi imitation. Immunohistochemical pathology diagnosis [M]. Beijing: Beijing Science and Technology Press, 2007.

2. Wang Shuqin, Huang Yunchang, Li Hongfen, Ji Xiaolong. Application of immunohistochemistry in routine pathological diagnosis [J]. Chinese Test Diagnosis Science, 1998,2 (6): 315-316.

3. Zhili, Jia Junle. Application of antigen repair in immunohistochemical technology [J]. Baotou Medical College, 2006,22 (02): 204-205.

4. Chief Editor of Tang Weiguo. Preparation and Application of Medical Laboratory and Diagnostic Reagents [M]. Shanghai: Shanghai Science and Technology Academic Press, 1996.1214 5. Xu Liangzhong, Yang Wentao. Criteria for the determination of the immunohistochemical reaction results [J]. Chinese Journal of Cancer, 1996,6 (04): 229-231

[essential information]

Filing / manufacturer: Henan Sanot Biotechnology Co., LTD

Address: No.109, No.1, Cuizhu Street, Zhengzhou High-tech Industrial Development Zone

Contact information: 0371-56596916

Name of after-sales service unit: Henan Senot Biotechnology Co., LTD

Contact information: 0371-56596916

Production address: Building Area F, No.55, Yulan Street, Zhengzhou High-tech Industrial Development Zone

Production Record Certificate No.: Yuzhou Food and Drug Administration Equipment Production No.20140010 (more)

[Production date and service life] See the label for details

[Medical Device Record Certificate No. / Product Technical Requirements No.] Yu No.20180281

[Date of manual approval] August 10,2018

[Date of instruction modification] April 27,2020