EBER Probe/CD3 Antibody Dual Staining Kit
Instruction For Use

[Product Name]

EBER Probe/CD3 Antibody Dual Staining Kit

[Specification]

25 Tests/box, 50 Tests/box.

[Intended Use]

This kit is used for in situ hybridization (ISH) and immunohistochemical staining based on HE staining to provide auxiliary information for physicians in diagnosis.

This kit is used to detect E-B virus encoded RNA (EBER) by in situ hybridization (ISH) in formalin-fixed paraffin-embedded tissue sections, which can provide auxiliary information for the diagnosis in clinic.

CD3 antibody was used for the qualitative determination of CD3 antigen in buffered formalin fixed paraffin-embedded tissue section cells.

[Principle of Detection]

EB virus expresses two small RNAs that do not translate into proteins (EBER, EBER1/EBER2), and the primary role is to inhibit interferon-mediated antiviral effects and apoptosis. EBER abounds in EBER infected cells, each cell can reach 10^6-10^7 copies, therefore, Specific oligonucleotide probe designed according to EB virus sequence can combined with EB virus transcription EBER. Through the immunological antigen antibody specificity combined, and redox chromogenic reaction, display the intracellular antigens, and carries on the localization, the qualitative, so as to test the EB virus's transcription or proliferation.

[Main Components]

Mainly including digoxin labeled EBER probe, anti-digoxin antibody, mouse anti-human CD3 immunohistochemical monoclonal antibody, pepsin working liquid, endogenous peroxidase blocking reagent, DAB Enhancer, post-linker reagent, polymer, DAB substrate, DAB buffer, HRP-Green substrate, HRP-Green buffer, PBS powder, Tween-20, Nuclear fast red.

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

EDTA antigen retrieval buffer .

EBER positive contrast photo (lymphoma); Xylene; Ethanol (anhydrous, 85%, 70%); Purified water; Clear-mount ; Rubber cement.

[Storage and Validity]

Stored at 2 ~ 8℃, valid for 12 months.

The kit is ready-to-use. After each use, the kit should be immediately put back to the refrigerator at 2 ~ 8℃ for preservation and finished within six months.

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

[Specimen Requirements]

Tissue samples of lymphoma fixed with formalin for 8~24h, then collected, dehydrated, embedded in paraffin, and made into paraffin blocks according to the pathological technical specifications. Paraffin block should avoid contacting with acid, strong alkali, and overheating, paraffin block should be stored in ventilated, dry paraffin block cabinet.

Tissue sections with a thickness of 3 to 5 μm were attached to adhesive slides. Removed the Water in the tissue sections and then placed it in an incubator at 60℃ (±5℃) for 30min to 2h or overnight at 37℃. Take the tissue sections out from the incubator and cooled at room temperature. If the tissue sections are stored at 2 ~ 8℃, the test should be completed within 3 days.

[Test Method]

Device and equipment required for testing

Pipette, in situ hybridization system, optical microscope, pressure cooker, timer, staining rack, wash bottle, incubation box.

Preparation of solution

1. Washing solution: Dissolve PBS powder into purified water, dilute the volume to 2L, add Tween-20 at a ratio of 0.05% (V/V), and mix it upside down.

2. DAB chromogenic solution: it is prepared by 1:20 ratio of DAB substrate and DAB buffer solution.

3. HRP-Green chromogenic solution: it is prepared by 1:30 ratio of HRP-Green substrate and HRP-Green buffer solution.

Sample processing and section preparation

1. The sample was fixed in formalin buffer solution for 8-24h at room temperature. In order to achieve the best and uniform fixation and paraffin embedding effect, the sample size should not exceed 0.5cm^3.

2. Penetration and embedding shall be carried out at a temperature lower than 65℃.

3. Slide the slides onto sticky slides.

operation steps

1. Preprocess tissue sections

1.1 Bake slices at 60-65℃ for about 2 h for fixation.

1.2 Room temperature deparaffinization in xylene for 3 times, 10 min/ time.

1.3 Soak into anhydrous ethanol twice, 5 min/ time.

1.4 Dry the tissue slices at room temperature for 5-10min.

1.5 Place the sections in an environment of 37℃, and add pepsin working solution to completely cover the sample tissues, the digested for 10-20 minutes.

Note: Digestion time varies according to different tissue types and slice thickness.

1.6 soak in deionized water for 2min.

1.7 Process the tissue sections by 70%, 85%, anhydrous ethanol in sequence for 2 min respectively at room temperature.

2. Specimen hybridization

2.1 Remove the EBER probe from the refrigerator, centrifuge instantly, and add 10 μL to the tissue area with a pipette.

2.2 Cover the specimen with coverslip to avoid bubbles, Use rubber cement to seal around the cover glass.

2.3 the sections were denatured at 70 ° c for 8min and then hybridized overnight at 37 ° c.

3. Wash after hybridization

3.1 Carefully remove the rubber cement and dip the glass into the washing solution at room temperature until the coverslip slide down.

3.2 Dip in washing solution for 3 times, 2 min/ time.

4. Incubate antibodies and Chromogen

4.1 Add 70-100μl anti-digoxin antibody

4.2 Incubate at room temperature for 40-60 min.

4.3 Soak in the washing solution for 3 times, 2 min/ time.

4.4 Add 70-100μl post-linker antibody reagent and incubate at room temperature for 20 min.

4.5 Dip in the washing solution for 3 times, 2 min/ time.

4.6 Add 70-100μl polymer drops and incubate at room temperature for 20 min.

4.7 Soak into the washing solution for 3 times, 2 min/ time.

4.8 Soak into deionized water once, 2 min/ time.

Note: To ensure adequate elution, the slides can be pulled several times during soaking

4.9 Drop 70-100μl freshly prepared DAB chromogenic solution according to tissue size.

4.10 Incubate at room temperature for 3-5 min.

4.11 Rinse with purified water.

4.12 Soak into the washing solution for 3 times, 2 min/ time.

5. Antigen retrieval and Chromogen

5.1 To heat EDTA antigen retrieval buffer in the pressure cooker to boiling. The slices were placed into the pressure cooker. Continue heating for 10min.

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

5.2 Remove the pressure cooker from heat source and cools down naturally. Remove the slice out from liquid in the pot after it cools down to room temperature naturally.

5.3 Soak into purified water for 3 times, 2 min/ time.

5.4 Soak into the washing solution for once, 2 min/ time.

5.5 Add endogenous peroxidase blocking reagent and incubate at room temperature for 5 min.

5.6 Soak into the washing solution for 3 times, 2 min/ time.

5.7 Drop 70-100μl CD3 antibody

5.8 Incubate at room temperature for 40-60 min.

5.9 Soak into the washing solution for 3 times, 2 min/ time.

5.10 Add 70-100μl post-linker antibody reagent and incubate at room temperature for 20 min.

5.11 Soak into the washing solution for 3 times, 2 min/ time.

5.12 Add 70-100μl polymer drops and incubate at room temperature for 20 min.

5.13 Soak into the washing solution for 3 times, 2 min/ time.

5.14 Drop 70-100μl freshly prepared HRP-Green chromogenic solution according to tissue size.

5.15 Incubate at room temperature for 3-5 min.

5.16 Rinse with purified water, then soak into purified water for 2 min.

5.17 Add DAB Enhancer and incubate at room temperature for 5 min.

5.18 Soak into the washing solution for 3 times, 2 min/ time.

5.19 Add 100μl nuclear fast red to the tissue area to be tested and incubate for 2-5 min.

Note: depending on the strength of the active nuclear fast red solution and the incubation time, may result in a reaction of pink in the nucleus, contrast staining results, while overstain or under stain may lead a mistake to the judgment of results.

5.20 Add appropriate clear-mount to seal and store.

Note: Do not use ethanol for dehydration and transparent xylene for tissue slices. For long-term storage, the clear-mount can be sealed with neutral balsam after the clear-mount has solidified.

【Quality control】

1. Positive control

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

Each stain 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.

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.

[Positive judgment value]

1. The staining results shall be based on the establishment of the positive tissue control and the blank control experiment. The interpretation of the staining results shall be positive (+) or negative (-).

1.1 Positive staining means that there is brown staining in the nucleus of specific cells and green in the cell membrane of specific cells in the tissue section, and no background staining.

1.2 Negative staining results showed that no brown or green staining in the expected nucleus or cell membrane of the tissue.

2. Based on the establishment of the positive and blank control experiments, positive staining in the tested tissues indicated expression EBER and the existence of CD3 antigen in the tissue sections.

Note: During each staining, positive and blank controls must be used, or the results cannot be used.

3. Based on the positive tissue control and blank control experiments, negative staining in the tested tissue slices indicates that EBER is not expressed and CD3 antigen is not existed in the tissue sections.

4. If both the positive and blank control tests are negative, indicating reagent failure or test operation error, the test shall be retested, and the quality control of the operation process and test results shall be carried out.

[Interpretation of results]

1. In each experiment, positive control and blank control must be carried out simultaneously.

2. If a positive control does not show an appropriate positive stain, the test for that batch of samples shall be deemed invalid.

3. If the incubation temperature of anti-digoxin antibody is too high or the incubation time is too long, it may lead to excessive staining and background staining.

[Method Limitations]

1. This product belongs to in vitro diagnostic reagent. This product is only used for EBER and CD3 antibody detection, not for other types of detection.

2. This antibody must be used by highly trained professionals.

3. A negative result does not necessarily mean that no EBER is present, may be the consequence of the unstable tissue fixation, poorly dehydrated, too old tissue, or poorly digested.

4. Mouse anti-human CD3 immunohistochemical monoclonal antibody is derived from biological resources, and its treatment should comply with relevant laws and regulations.

5. The reagent shall be protected with appropriate measures to avoid contact with the skin and eyes.

6. Whether this reagent is applied to non-formalin fixed tissues has not been confirmed.

[Performance]

1. Staining intensity

The nucleus of specific cells in the tissue section have brown coloration and the cell membrane of specific cells have green coloration, and no background staining.

2. Consistency

Base on the establishment of the blank control, after the EBER probe and CD3 antibody were incubated with the positive control and the negative control, respectively, and observed under a optical microscope, only the positive control sample staining results were positive, and the blank control and negative control staining results were negative.

3. In-batch repeatability

Stain sections from the same positive tissue with the same batched reagent, with no significant difference in staining intensity or localization.

4. Batch repeatability

Staining of sections from the same positive tissue with different batches of reagent does not differ significantly in the intensity or location of the staining.

[Cautions]

1. To avoid contact with skin or eyes, appropriate protective measures should be taken.

2. DAB in staining solution is a carcinogen, appropriate protective measures should be taken during operation.

3. Expired reagents may be less active, so do not use expired reagents.

4. If the components of this product are mixed with products of other companies, abnormal conditions may occur during the staining process.

5. Waste generated during usage shall be disposed of accordance to medical waste management regulations.

[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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