EBER Probe (in situ hybridization)
Instruction For Use

[Product Name]

EBER Probe (In Situ Hybridization)

[Specification]

50 Tests/box, 100 Tests/box.

[Intended Use]

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.

[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]

NO.	Reagent	Storage Temperature	Specification	
			50tests	100tests
1	EBER probe	4℃	14ml	28 ml
2	peroxidase blocking reagent	4℃	11ml	20 ml
3	anti-digoxin antibody	4℃	11ml	20 ml
4	Post-linker reagent	4℃	11ml	20 ml
5	polymer	4℃	11ml	20 ml
6	DAB substrate	4℃	3ml	3 ml
7	DAB buffer	4℃	30ml	40 ml
8	pepsin working liquid	4℃	14ml	28 ml
9	DAB Enhancer	4℃	9ml	20 ml
10	hematoxylin	4℃	11 ml	20 ml
11	EBER positive control slide	4℃	1pcs	1pcs
12	Instruction for use	/	1pcs	1pcs

[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.

[Applicable Instrument]

IHC automated system.

[Specimen Requirements]

Tissue samples of nasopharyngeal cancer, gastric cancer or 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 30 to 60 minutes or overnight at 37℃. Take the tissue sections out from the incubator and cooled at room temperature. If the tissue sections are stored at room temperature, the test should be completed within 7 days. If the tissue sections are stored in cold storage (2 ~ 8℃), the test should be completed within 1 month.

[Test Method]

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 on the IHC automated system

1. Bake slices at 60-65℃ for about 1-2 h for fixation.(You can choose to bake slices on the IHC automated system).

2. Add reagents

2.1 Use the code scanner to scan the secondary antibody strip barcode, and click OK.

2.2 Add DAB Enhancer reagent manually.

2.3 Use the scanner to scan the side code of the probe strip bottle (when scanning pepsin, please manually select *Enzyme 1 or *Enzyme 2 or other, and manually enter the time and date such as 2020-11-11; when scanner scans DAB Enhancer, please manually select DAB Enhancer and enter the time and date (such as 2020-11-11), click OK; click Add reagent, select the reagent type “ probe”, select the preferred status “all”, and check *EBER Probe as Preferred.

3. Set program

3.1 Select pre-staining; Select in situ hybridization; Modify denaturation and hybridization conditions (denaturation at 70℃ for 8min; hybridization at 37℃ for 2h).

3.2 Select staining; Select in situ hybridization; Modify the running program (blocking for 5min, primary antibody for 40min, post-linker for 20min, polymer for 20min, DAB chromogen for 2

min, DAB Enhancer treatment for 5min (optional), hematoxylin counterstaining for 30s (It can be modified appropriately according to the counterstaining habits).

4. Set slide

Add case; Add slide; Select in situ hybridization; Select *EBER Probe; Select staining program (modify as step 3.2 of setting program), select enzyme repair time ≥10 min and <15 min (scan pepsin with the scanner) When manually selecting the same name, it is recommended to copy from *Enzyme 1 10min and modify it); Select denaturation/hybridization conditions (modify as step 3.1 of setting program); Add slides; Print slide labels, and run on the machine (Note : No hot repair process).

Note: Once the machine starts to run, do not stop midway until the end of the run.

5. Gradient ethanol (70%, 85%, 100%) dehydration, xylene transparent, Neutral balsam sealed, reading.

【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 yellow or brownish yellow staining in the nucleus of specific cells in the tissue section, and no background staining.

1.2 Negative staining results showed that no yellow or brownish yellow staining in the expected nucleus of the tissue.

2. Based on the establishment of the positive and blank control experiments, positive staining in the tested tissues indicated the existence of EB virus transcription and proliferation 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 E-B virus is not infected, or E-B virus is not transcribed 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 is only used for EB virus detection, not for other types of detection.

2. 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.

[Performance]

1. Staining intensity

The nucleus of specific cells in the tissue section have yellow or brown-yellow coloration and no background coloration.

2. Sensitivity of probe

Test EBER positive tissues from lymphoma, stomach cancer, or nasopharyngeal cancer, the results are all positive.

3. Probe specificity

After the probe hybridized with HPV positive, EBER positive, and HBV positive tissues respectively, it is observed under ordinary light microscope that only EBER positive tissue samples show positive cell staining, the HPV and HBV positive samples show no positive staining.

4. In-batch repeatability

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

5. Batch repeatability

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

[Cautions]

1. The product is only applicable to EBER in situ hybridization detection and not used for any other purposes.
2. Expired reagents may be less active, so do not use expired reagents.
3. If the components of this product are mixed with products of other companies, abnormal conditions may occur during the staining process.
4. 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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