Cytomegalovirus (CMV) DNA Probe (ISH)

Instruction For Use

[Product Name]

Cytomegalovirus (CMV) DNA Probe (In Situ Hybridization)

[Specification]

25 tests/kit, 50 tests/kit

[Intended Use]

Mainly used for the detection of cytomegalovirus in situ hybridization on paraffin sections. Provide clinical auxiliary information for physicians in diagnosis.

[Test Principle]

Mark the certain known nucleic acid sequence with digoxigenin to make probes. The probes are hybridized with nucleic acids in tissue sections to form double-stranded nucleic acid molecules. Then the nucleic acid target-antibody complexes are formed by specific binding of the antigen (digoxigenin molecule)-antibody (anti-digoxigenin antibody). After that, add post-primary antibody reagents and horseradish peroxidase HRP-labeled polymer in sequence to form a target-antibody complex. Finally, DAB is catalyzed by HRP to form a brown precipitate at the target position.

[Main Components]

Components	Main Compositions	Specifics	
		25 tests/kit	50 tests/kit
CMV probe	Probe, Formamide, Dextran sulfate	7 mL	14 mL
Anti-digoxigenin antibody	Anti-digoxigenin antibody, Antibody diluent	5 mL	9 mL
Pepsin solution	Pepsin	5 mL	9 mL
DAB Enhancer	Copper sulfate	5 mL	9mL

The reagent does not contain but necessary for the test: IHC reagents (Celnovte product No. CF10907; contains peroxidase blocking reagent; post-primary antibody reagent, polymer, DAB substrate, DAB buffer, hematoxylin); concentrated washing solution (10×) (Celnovte product No. SN640601).

Other reagents not provided: Xylene; Ethanol (anhydrous, 85%, 70%); Purified water; Neutral gum; Rubber cement.

[Storage and Validity]

Stored at 2℃~8℃ with a valid period of 12 months.

Shipping conditions: The shipping temperature should not exceed regular room temperature, and the shipping time should not exceed one week.

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

[Applicable Instrument]

Immunohistochemical staining machine.

[Sample Requirements]

Fresh biopsy or surgical samples of lung, liver or stomach, etc., are 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 avoid contact with acid and strong alkali, and avoid overheatingbe, and should be stored in a special, ventilated and dry paraffin block cabinet.

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℃ (±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. If the tissue sections are in cold storage (2 ~ 8℃), the detection should be completed within 3 months.

[Test method]

1. Instruments and equipment required for testing

Immunohistochemical staining machine, microscope.

2. Solution preparation:

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

3. Sample Handling and Slide Preparation

3.1 Fix the sample in formalin buffer for 8-24 hours at room temperature. In order to achieve the best and uniform fixation and paraffin embedding effect, the sample size should not exceed 0.5cm³.

3.2 Infiltration and embedding should be performed below 65°C.
3.3 Sections were removed from adhesive glass slides.
4. Test procedure
4.1 Baking slices: 60-65 ℃ baked slices fixed for about 1-2 hours (it also works to bake slices on other machine, such as Leica automatic IHC dyeing machine).
4.2 Adding reagents: Refer to the instructions on adding reagents in automatic IHC staining machine guide manual.
4.3 Setting up procedure:
4.3.1 Denaturation and hybridization conditions
Denaturation: 70℃, 8min;
Hybridization: 37℃, 2h.
4.3.2 Procedures
Peroxidase blocking reagent for 5min,
Anti-digoxigenin antibody for 40min,
Post-primary antibody reagent for 20min,
Oolymer for 20min,
DAB chromogenic solution (DAB substrate and DAB buffer in a ratio of 1:20)) for 2-5 min of color development,
DAB Enhancer treatment for 5 min (optional),
Hematoxylin counterstaining for 20s (which can be modified according to the habit of backing staining).
4.4 Slide setting: Repair with pepsin solution for 15min.
4.5 Dehydrate with graded ethanol, transparent with xylene, and mount.
4.6 Reading.

[Quality control]
1. Positive Control
A positive control serves as an indicator of proper tissue preparation and proper staining technique. Each staining should include a positive pair of photographs for the same test condition for comparison. Known positive tissue controls can only be used to monitor the proper execution of steps and testing of reagents, and are not intended to help describe a definitive diagnosis on patient samples. If the positive tissue control fails to show appropriate positive staining, the results of the batch of experimental test samples should be considered invalid.
2. Blank control
Each staining should include a blank control reagent under the same test conditions for comparison. The blank control reagent is used instead of the product to stain tissue

sections, which is used to judge non-specific staining and provide a better explanation for specific staining.

[Positive judgment value]

1. The staining results must be based on the establishment of the tissue positive control and blank control experiments. The interpretation of the staining results: positive (+)/negative (-).
1.1 Positive staining results refer to brown staining in the cytoplasm of specific cells in the tissue section without background staining.
1.2 A negative staining result is that no brown staining is seen in the expected cytoplasm in the tissue.
2. Based on the establishment of the positive and blank control experiments, the positive staining in the tested section indicates the presence of cytomegalovirus infection on the tissue slices.
Note: During each staining process, there must be positive control sections and blank control reagents, otherwise the results cannot be used.
3. Based on the establishment of the positive and blank control experiments, the negative staining in the tested section indicates that there is little or low possibility of cytomegalovirus infection on the tissue slices.
4. If the positive control does not show the appropriate positive staining, it indicates an operational error or the reagents are invalid. This batch of samples should be re-tested, and the quality control should be carried out on the operation process and test results.

[Results Interpretation]

1. In each experiment, there must be a positive control at the same time.
2. If the positive control fails to show proper positive staining, the test of the batch of samples should be judged invalid.
3. If the anti-digoxigenin antibody incubation temperature is too high or the time is too long, it may lead to strong staining and background staining.

[Limitations of detection method]

1. This product is only used for cytomegalovirus detection, not for other types of detection.
2. A negative result refer that CMV RNA is not detected, but it does not mean that CMV RNA is not present in the sample. Poor tissue fixation, poor dehydration,

long-term storage of tissue, and improper enzyme digestion, all of which would cause RNA to fail to be detected.

[Product performance index]

1. Staining intensity

Specific cells in tissue sections got brown staining in the cytoplasm and no background staining.

2. Probe Sensitivity

CMV-positive tissues such as lung, liver, or stomach were tested and got all positive staining results .

3. Probe specificity

After the probes were hybridized with HPV-positive, CMV-positive, and HBV-positive tissues, respectively, and observed under an ordinary light microscope, only CMV-positive tissue samples showed positive cell staining, while HPV-positive and HBV-positive samples had no positive cell staining.

4. In-batch repeatability

The same batch number of reagents stained CMV-positive tissue sections from the same tissue source. There was no significant difference in staining intensity and location of tissue slices.

5. Inter-batch repeatability

Different batches of reagents stained CMV-positive tissue sections from the same tissue, and there was no significant difference in the intensity and localization of the staining.

[Cautions]

1. This product is only ued for CMV in situ hybridization detection, not for other purposes.
2. Appropriate protective measures should be taken to avoid contact of the reagent with skin or eyes.
3. DAB in the dyeing solution is a carcinogen, and appropriate protective measures should be taken during the operation.
4. The activity of the reagents beyond the expiration date may be reduced, so do not use expired reagents.
5. If the components of this product are mixed with other company's products, abnormalities may occur during the dyeing process.
6. The waste generated during use shall be disposed of in accordance with the Regulations on the Management of Medical Waste.

[References]

1. Greenaway PJ, Wilkinson GW. Nucleotide sequence of the most abundantly transcribed early gene of human cytomegalovirus strain AD169. Virus Res, 1987, 7: 17-31.
2. Giovanna Bergamini, Marko Reschke， et al. The Major Open Reading Frame of the β2.7 Transcript of Human Cytomegalovirus:InVitro Expression of a Protein Posttranscriptionally Regulated by the 5′Region.Journal of Virology ,1998, 72(10):8425.
3. Brian P. McSharry,1Peter Tomasec,et al.The most abundantly transcribed human cytomegalovirus gene (b2.7) is non-essential for growth in vitro.Journal of General Virology (2003), 84, 2511–2516.

[Basic Information]

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