



RISH™ Cytomegalovirus (CMV) Probe
Hybridization Probe

ISO
9001:2000
CERTIFIED

Control Number: 902-RI0011-090310

Catalog Number: RI0011T

Description: Approximately 20 tests at 20 microliters per test

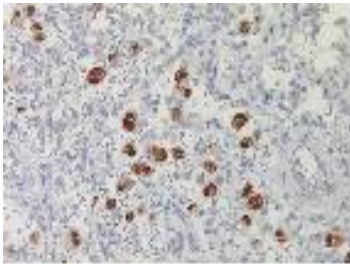
Intended Use:

For Research use only. Not for use in diagnostic procedures.

The Cytomegalovirus (CMV) digoxigenin labeled probe is intended for the detection of CMV DNA and β 2.7 RNA in tissues and cells infected by the human Cytomegalovirus.

Summary & Explanation:

CMV is a member of the human herpes virus-5, HHV-5 group and can be transmitted via breast milk, during organ transplantation, sexual activity or during blood transfusions. It is estimated that 40-100% of people may be infected with this virus. CMV infections are common causes of morbidity and mortality especially in immune compromised individuals. Biocare's CMV digoxigenin labeled probe is intended for the detection of CMV genomic DNA and RNA in tissues and cells. The detection is carried out by *in situ* hybridization in formalin-fixed paraffin -embedded (FFPE) histological sections. In addition to the detection of CMV at the DNA level, the CMV probe will also hybridize to the β 2.7 RNA, which is expressed during all stages of infection. The *in situ* hybridization technique offers an important advantage over immunohistochemistry, as it virtually lacks background, and allows a clean and sharp viewing of the histological preparation.



Human lung stained with RISH™ CMV probe

Principle of Procedure

This digoxigenin-labeled DNA probe will hybridize to its specific CMV DNA / RNA target in cells infected by the human Cytomegalovirus. The labeled probe is detected with an unconjugated anti-digoxigenin antibody, followed by a polymerized HRP incubation step. The DNA probe is indirectly evidenced by a colorimetric reaction.

Known Applications:

in situ hybridization (formalin-fixed paraffin-embedded tissues).

Supplied As:

RTU DNA probe in buffered formamide with nucleic acid carriers.

Materials and Reagents Needed But Not Provided:

RISH™ Detection Kit (RI0207KG)*

Decloaking Chamber™ (pressure cooker)*

RISH™ Retrieval Solution (RI0209M)*

IQ Kinetic Slide Stainer* or other hybridization oven

IQ Aqua Sponge*

Positively charged microscope slides

Desert Chamber* (drying oven)

Positive and negative tissue controls

Xylene (could be substituted with xylene substitute)

Ethanol or reagent alcohol

Deionized or distilled water

Material and Reagents Needed But Not Provided cont'd:

TBS Wash Buffer (TWB945)*

Hematoxylin*

Bluing Reagent*

Mounting medium*

Peroxidase*

HybriSlip™ (or equivalent)*

Thermal Test Strips*

* Biocare Medical Products: Refer to a Biocare Medical catalog for further information regarding catalog numbers and ordering information. Certain reagents listed above are based on specific application and detection system used.

Species Reactivity:

Human Cytomegalovirus DNA / RNA in tissues and cells.

Cellular Localization: Nuclear and/or cytoplasmic

Storage and Stability:

Store probe at 2°C to 8°C. Do not use after expiration date printed on vials. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

Protocol Recommendations:

Refer to RISH™ Detection Kit (RI0207KG) data sheet for specific protocol recommendations.

Technical Notes:

This test should be performed on tissue sections where the presence of Cytomegalovirus is anticipated. 4-5 micrometer (μ m) sections are sufficient to conduct this study. Preferably, the sections should be fresh and no more than 30 days old.

This DNA probe has been standardized using Biocare's IQ Kinetic Slide Stainer for Denaturation (95°C), hybridization (37°C) and post-hybridization detection steps. Detection steps can also be programmed on an automated staining system.

If using IQ1000 (single hot bar) place slides onto rack and denature on hot bar at 95°C for 5 minutes. After denaturation, remove rack and place on bench. Turn off hot bar and unplug unit. Cool hot bar (3-5 minutes) with running tap water until bar approximates 35-40°C. Re-set hot bar to hybridization temperature (37°C). Place water-saturated IQ Aqua sponge and a thermometer onto hot bar before hybridization. Check the temperature on the hot bar. It should not be higher than 40°C. Place rack with slides onto sponge, cover unit and incubate for 1 hour.

If an IQ Kinetic slide stainer is not available, consider using a hot plate to denature probe / slide at 95°C for 5 minutes. After denaturing slide, use a commercially available humidity chamber for hybridization at 60 min. Both incubator and humidity chamber must be at 37°C when hybridizing probe. Other hybridization chambers can be used, but measures should be taken to ensure that chamber is hermetically sealed during hybridization.

*If a Decloaking Chamber or pressure cooker is not available, consider using a water bath or hot plate for retrieval. Place RISH™ Retrieval (1X) in glass (Pyrex) container and heat solution until the appropriate temperature is achieved (90°C).

Heat slides in this solution for 15 minutes. Remove slides after incubation, allow to cool, and wash in distilled water prior to detection steps.

**The IQ Stainer can be used as an incubation and humidity chamber by using the IQ Aqua Sponge. Saturate IQ Aqua Sponge with distilled water, and place on hot bar set to 37°C for hybridization. Use the clear plastic hood to contain heat and moisture.

If probe appears cloudy, briefly vortex and heat to hybridization temperature 37°C before application.

The use of probe in amounts less than recommended may lead to inconsistent results.





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Quality Control:

Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information on tissue controls.

Precautions:

This hybridization probe contains formamide in concentrations and volumes that are harmful to health. Avoid any direct contact with reagents. Take appropriate protective measures (use disposable gloves, protective glasses, and lab garments).

Troubleshooting:

Follow the reagent specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

Troubleshooting Guide:**No Staining**

1. Critical reagent (such as probe) omitted
2. Incorrect denaturation / hybridization temperature (less than 95°C / 37°C) used
3. Staining steps performed incorrectly or in the wrong order
4. Low or compromised target DNA / RNA
5. Detection reagent incubations too short
6. Improperly mixed substrate and/or chromogen solution(s)

Weak Staining:

1. Tissue is either over-fixed or under fixed
2. Denaturation / hybridization temperatures incorrect.
3. Probe incubation time too short
4. Low expression of RNA, contamination of tissues with RNases or RNA degradation
5. Compromised genomic or target DNA
6. Over-development of substrate
7. Omission of critical reagent (digestion or retrieval solution)
8. Incorrect procedure in reagent preparation
9. Improper procedure in steps
10. Incorrect hybridization temperature (greater than 37°C) used

Non-specific or High Background Staining

1. Variable fixation time
2. Substrate is overly developed
3. Tissue was inadequately rinsed
4. Deparaffinization incomplete
5. Tissue damaged or necrotic
6. Sections dried during hybridization

Tissues Falling off Slide

1. Slides were not positively charged
2. A slide adhesive was used in water bath
3. Tissue was not dried properly
4. Tissue contained too much fat
5. Tissue may be over digested

Specific Staining too Dark

1. Incubation of probe, secondary or tertiary too long

Performance Characteristics:

The optimum parameters and protocols for a specific application can vary. These include, but are not limited to: fixation, enzymatic digestion, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended hybridization and incubation times listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

Limitations & Warranty:

There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by this product.

References:

1. Scheurer ME, Bondy ML, Aldape KD, Albrecht T, El-Zein R. Detection of human cytomegalovirus in different histological types of gliomas. *Acta Neuropathol.* 2008 Jul;116 (1):79-86.
2. Cobbs CS, Soroceanu L, Denham S, Zhang W, Kraus MH. Modulation of oncogenic phenotype in human glioma cells by cytomegalovirus IE1-mediated mitogenicity. *Cancer Res.* 2008 Feb 1;68(3):724-30.
3. Harkins L, Volk AL, Samanta M, Mikolaenko I, Britt WJ, Bland KI, Cobbs CS. Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. *Lancet.* 2002 Nov 16; 360(9345):1557-63.
4. Cobbs CS, Harkin L, Samanta M, Gillespie GY, Bharara S, King PH, Nabors LB, Cobbs CG, Britt WJ. Human cytomegalovirus infection and expression in human malignant glioma. *Cancer Res.* 2002 Jun 15;62(12):3347-50.

