

 VITRO S.A
Calle Luís Fuentes Bejarano 60
Edificio Nudo Norte Local 3
41020 Sevilla (España)
F: +34 954 922 892
T: +34 954 933 200
www.vitro.bio

Epstein Barr Virus (EBER) CISH Detection Kit (Incl. Detection system)

DESCRIPTION

This kit contains reagents to perform manual or automated chromogenic in situ hybridization (CISH) technique on human tissue sections fixed in buffered formalin and embedded in paraffin.

This kit contains a visualisation system based on the Master Polymer Plus HRP detection system and is sufficient to perform 20 determinations following the recommended protocol.

Presentation ▽: The general reference / presentation for this kit is:

MAD-001897QK 20 tests

This reference is for presentation packaging in Low Density Polyethylene (LDPE) dropper. In case the user wants other presentations (references / different volumes) must contact the supplier.

Intended Use : Diagnostic in vitro in humans

Storage conditions : refrigerator between 2 and 8 ° C.

Warranty : The container once opened the reagent can be used until the expiration date indicated on the label. If the reagent has been stored under conditions other than those indicated in this document, the user must previously check its correct functionality considering that the product's warranty is no longer valid.

Special Handling Instructions: This reagent is specially designed for handling in Lab Vision Autostainer 480 and 780.

Warnings and Precautions: 1) The product may only be operated by trained users and authorized laboratories. 2) Please note that the ultimate responsibility in the optimization and interpretation of chromogenic hybridizations technique corresponds to the attending physician and technicians who use the kit. Also, this set of reagents is only a useful tool for the interpretation of morphological findings of each case in conjunction with other relevant diagnostic tests and patient's clinical data.

3) The reagent contains sodium azide (NaN₃) as a preservative. Although this product is highly toxic and if mixed with water or acids, mainly in the presence of metals there is danger of explosion, these risks are minimized to the maximum when used at concentrations below 0.05% as in this case. However, for handling this reagent the following precautions should be taken: a) Use of gloves and protective equipment established for hybridization and immunohistochemical techniques and lab strict compliance with the general safety practices existing in it; b) Do not store reagents in metal packaging and do not use metal tools for its handling c) Store waste for disposal in appropriate containers regulated under current regulations in each laboratory.

SPECIFICITY

The Epstein-Barr virus (EBV) also called human herpes virus type 4, which was discovered by Epstein, Achong and Barr in 1964 in cell cultures of Burkitt's lymphoma, is a virus of the herpes family of 120-180 nm in diameter composed of a double-stranded DNA of 192,000 bp containing 85 genes. It is one of the virus with the major capacity of infection in humans in which, once incorporated persists throughout the individual's life with a prevalence of 90% in adults. Structurally, the viral genome is contained in a nucleocapsid that in turn, is surrounded by a protein coat containing a lipid component and the glycoprotein gp350.

Like other herpes virus, EBV infects cells that are not actively dividing, in this case resting mature B lymphocytes. For this, the gp350 envelope virus initiates adhesion to the surface of the host cell by binding to

the CR2 molecule (CD21), which facilitates activation of the glycoprotein gp42 which, together with the HLA class II and gp25 and gp85 proteins of the virus form a complex which is used for penetration on B lymphocytes. Once the virus enters the cell, virus capsid dissolves and the viral genome is transported to the nucleus where it is inserted by the action of DNA polymerase of the own host cells.

Once in the nucleus of the host cell (usually the memory B cells), the virus enters in a period of latency during which no new virions are produced and expresses only a limited number of their genes (about 11) encoding various molecules. Among them there are two types of messenger non-coding RNA, six nuclear proteins and two membrane proteins. This remarkable surface protein expression makes difficult the recognition by cytotoxic T cells of the infected cells.

EBV nuclear antigen (EBNA) 1 is the protein that allows the virus to remain within the B cell as a circular DNA episome. By contrast, the antigen EBNA 2 regulates the expression of latent membrane protein (LMP) 1 and 2 of the EBV as well as the proteins contributing to the growth and transformation of B lymphocytes.

LMP-1 protein acts as an oncogene that mimics the function of the CD40 molecule on the surface of the lymphocytes and is able to bind to various receptors of tumor necrosis factor, resulting in the activation of nuclear factor *κB* and *c-jun* and numerous other cell adhesion molecules; this way EBV enhance the cytokine production capacity and generates a powerful cellular proliferation signal. Additionally, LMP-2 EBV protein, which prevents EBV reactivation from latency stage by blocking the phosphorylation of tyrosine kinases, guarantees the persistency of a sufficient population of infected cells to ensure the survival of the virus. EBER 1 and 2 RNAs of EBV, which do not encode proteins and therefore cannot be transcribed, play an important role in oncogenesis and the resistance of infected cells to programmed cell death and apoptosis. EBER genes are transcribed by the viral RNA polymerase and are most abundant EBV molecules in Burkitt's lymphoma and other latently infected cells. Functionally the type EBER RNAs act as transcription factors of interleukin-10 which is perhaps the most important existing autocrine growth factor in Burkitt lymphoma.

At certain times, or only in certain patients from the beginning, the virus can enter a lytic phase or of productive infection that results in the generation of large numbers of infectious virions. This requires that the virus genome passes from circular to linear with the early activation of many genes, including the viral DNA polymerase itself.

Primary EBV infections usually occur in the childhood and are asymptomatic or cause mild nonspecific symptoms. On the contrary the adolescent's and adult's infections quite often cause infectious mononucleosis, which can occasionally cause serious complications such as haemolytic anaemia, thrombocytopenia, myocarditis, hepatitis, splenic rupture, or neurological complications such as Guillain-Barré syndrome or encephalitis. In these cases the virus is transmitted through saliva and initially infects epithelial cells of the oropharynx where it replicates until infects B lymphocytes. Furthermore, dormant infected B-lymphocytes spread throughout all the lymphopoietic organs, which are the source of continuous propagation of the virus. Even if normally the infection is controlled by cytotoxic T cells, in patients with X-linked lymphoproliferative EBV infection may be fatal.

The digoxigenin-labeled probe included in this kit is a mixture of 5 RNA oligonucleotides complementary to type 1 and 2 EBER of EBV and has been created for the detection of the presence of EBV-infected cells in a latent stage. However, its use also produce tissue hybridization signals with EBV lytic infections.

DIAGNOSTIC APPLICATIONS

EBV is directly associated with various human cancers of epithelial, mesenchymal and especially lymphoid origin inside whose cells can be identified. Classic examples of related lymphoid malignancies, in many of which the determination of the EBV EBER1 RNA is diagnostic, are the classic type of Hodgkin's disease, the Burkitt lymphoma endemic type, plasmablastic lymphoma, some NK/T cell lymphomas and T lymphoproliferative disorders that appear in transplanted and immunocompromised patients such as those infected by the HIV virus.

Epithelial neoplasm

- Nasopharyngeal Carcinoma
- Gastric Carcinoma
- Medullary carcinoma of the pancreas
- Lymphoepithelial carcinomas (salivary glands, thymus, lung, breast, etc..)

Mesenchymal neoplasm

- Follicular dendritic cell sarcoma
- Leiomyomas / leiomyosarcomas in immunocompromised patients

Lymphomas

- Hodgkin lymphoma classic type
- Endemic Burkitt Lymphoma
- Lymphomatoid Granulomatosis
- Pyothorax associated lymphoma
- Primary effusion lymphoma
- Nasal type NK / T peripheral lymphoma
- Some diffuse large B-cell lymphomas
- T-cell rich B cell lymphoma
- Angioimmunoblastic T cell lymphoma

Lymphoproliferative disorders in immunocompromised patients

- Associated with primary immunodeficiency
- Associated with HIV infections
- Post-transplant lymphomas
- Methotrexate induced Lymphomas
- Large B-cell lymphoma of the elderly

PATTERN AND CONTROLS

Pattern: Nuclear

Positive Control: Tissue section from classic Hodgkin lymphoma or EBV-positive nasopharyngeal carcinoma.

Negative Control: Homologous preparation to the sample to be tested incubated with a nonspecific mRNA for EBV.

KIT LIMITATIONS

The use on frozen tissue has not been evaluated.

The kit is not recommended for use in decalcified samples. Nonspecific staining in the myeloid series has occasionally been detected in this type of sample, usually related to the inefficient blockade of endogenous peroxidase and / or to typical DNA and RNA degradation artifacts.

SAMPLE TYPES

Sections of 4 microns thick mounted on special slides for immunohistochemistry and obtained from paraffin-embedded tissues, preferably fixed in buffered formalin.

PRINCIPLE OF ANALYTICAL METHOD (in situ hybridization)

In situ hybridization (ISH) allows the detection of specific sequences of DNA or RNA in histological and cytological samples without losing morphological details. The ISH technique is based on hybridization between a DNA or RNA sequence specifically labelled (probe) and a sequence of DNA or RNA present in the sample. If there is any complementarity between the sequences the hybridization occurs, which results in a hybrid product. Hybrids can be easily viewed by a chromogenic immunohistochemical staining procedure (CISH) directed toward specific marker of the probe employed.

The ISH technique is highly sensitive, specific and easy to perform, and in addition, contains no radioactive products. The reagents supplied in this kit are perfectly matched and therefore, the kit is an easy to use tool to perform CISH.

COMPONENTS AND REAGENTS INCLUDED IN THE KIT:

MAD-001897QK			
MAD-001893QH Epstein Barr Virus (EBER1) CISH Kit			
Proteinase K Concentrated Solution (10 mg/mL)	MAD-001892QE	20 tests	200µl conc.
Epstein Barr Virus (EBER1) CISH PNA Probe	MAD-001893HS	20 tests	--
Digoxigenin (HY-A.1)	MAD-001892QD-4	20 tests	4 ml pred.
MAD-000237QK-10 Master Polymer Plus Detection System			
Peroxidase Blocking Reagent	MAD-021540Q-10	20 tests	10 ml
Primary Antibodies Amplifier Master	MAD-000237QK-B10	20 tests	10 ml
Master Polymer Plus HRP	MAD-000237QK-C10	20 tests	10 ml
DAB Substrate Buffer	MAD-001812QK-A	20 tests	15 ml
DAB Enhancer	MAD-001560Q-10	20 tests	10 ml
DAB Chromogen Concentrate	MAD-001812QK-B	20 tests	0,5 ml

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT

- Humid incubation chamber
- Slides treated with silane or electrically charged - **MAD-15-188-55/100**
- Coverslips
- Thermal plate or oven (37 ° C)
- Dewaxing and hydration battery (xylene, absolute ethanol and of 80% and 70% concentrations)
- TBS buffer - **MAD-004077R**
- Micropipettes
- Contrast haematoxylin - **MAD-108.1000**
- Optical microscope

TECHNICAL PROTOCOL FOR CISH EBER TISSUE USING MASTER POLYMER PLUS DETECTION SYSTEM

This protocol is preferred for conducting CISH on tissue sections fixed in buffered formalin and embedded in paraffin.

1. Dewax and rehydrate

- Incubate slides with paraffin tissue sections in the oven at 60 ° C overnight.
- Dewax:
 - Xylene 10 min 2x
- Rehydrate
 - Absolute ethanol 2x 5 min
 - Ethanol 80% 5 min
 - Ethanol 70% 5 min
 - Distilled water (10 min)

2. Enzymatic digestion

- Dilute 5 µl of concentrated **Proteinase K Concentrated Solution** in 2 ml of **TBS buffer**.
- Apply the resulting solution onto the tissue and incubate 8 min at room temperature (RT).
- Wash 3 times with TBS buffer at RT

3. Hybridization

- Apply 20 µl of **Epstein Barr Virus (EBER) CISH PNA Probe** on the tissue.
- Place a coverslip on the section.
- Place the slide into a wet camera and incubate 40min at 62°C.

4. Detection and visualisation (manual or automatic)

- a. Remove the coverslip and wash 3 times in TBS at RT
- b. Apply 200 µl of **Peroxidase Blocking Reagent** on the tissue and incubate 10 min at RT
- c. Wash 3 times in TBS.
- d. Apply 200 µl of **Digoxigenin (HY-A.1)** antibody on tissue and incubate for 10 min at RT.
- e. Wash 3 times in TBS.
- f. Apply 200 µl of **Primary Antibodies Amplifier Master** on the tissue and incubate 15 min at RT.
- g. Wash 3 times in TBS.
- h. Apply 200 µl of **Master Polymer Plus HRP** on the tissue and incubate 20 min at RT.
- i. Wash 3 times in distilled water
- j. Mix a drop of **DAB Chromogen Concentrate** with 1 ml of **DAB Substrate Buffer** and applying the resulting solution onto the tissue; incubate for 7 min at RT
- k. Wash 3 times in distilled water.
- l. Apply 200 µl of **DAB Enhancer** on the tissue and incubate 2 min at RT. (Optional)
- m. Wash 3 times in TBS.

5. Contrast staining and mounting

- a. Stain with **contrast haematoxylin**.
- b. Bluing in tap water.
- c. Dehydrate and clear with increasing concentrations of alcohols and xylene.
- d. Mount and interpret the results under a microscope.

INCIDENTS AND COMPLAINS

It is recommended to thoroughly follow all instructions contained in these technical data sheet. In case of occurrence of atypical or unexpected results please contact the Vitro SA sales representative of the area. If not, please contact Vitro S.A using its contact information as mentioned above.

SAFETY RECOMMENDATIONS

This product is intended for laboratory professional use only. The product is NOT intended to be used as a drug or for domestic purposes. The current version of the Safety Data Sheet for this product can be downloaded by searching the reference number at www.vitro.bio or can be requested at regulatory@vitro.bio.

LIMITATIONS OF THE REACTIVE

If you have met all the conditions of storage and handling in the laboratory, this reagent is guaranteed throughout its warranty life. Vitro S.A is not responsible for damage, personal injury or economic loss that this reagent can be involved.

REFERENCES

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LABEL AND BOX SYMBOLS

Explanation of the symbols of the product label and box:

	Health product for in vitro diagnosis.		Expiration date
	Catalog number		Temperature limit
	Lot code		Manufacturer
	Refer to the instructions of use		Sufficient content for <n> assays
	Material safety data sheet		