

Quantum EBV ISH Probe

QHD-042 Quantum EBV ISH Probe

Document Number: IFU-425_QHD-042 Quantum EBV Ish Probe
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Catalog Number	Description
QHD-042	This is a ready-to-use product. No reconstitution, mixing, or dilution is required. Differences in tissue processing and technical procedures in the laboratory may produce significant variability in results and consequently require regular use of controls (See Quality Control Procedures)

1. Intended Use

The Quantum EBV ISH Probe is intended to be used for the qualitative detection of human Epstein-Barr virus (EBV) EBER RNA in formalin-fixed, paraffin-embedded tissue by *in situ* hybridization (ISH) on the Quantum HDx stainer. The probe is intended to be used in combination with the Quantum Detection Kits.

2. Test Principle

The *in situ* hybridization (ISH) technique allows the detection and visualization of specific nucleic acid sequences in cell preparations. Hapten-labeled nucleotide fragments, so called ISH probes, and their complementary target sequences in the preparations are co-denatured and subsequently allowed to anneal during hybridization. Afterwards, unspecific and unbound probe fragments are removed by stringency washing steps. Duplex formation of the labeled probe can be visualized using primary (unmarked) antibodies, which are detected by secondary polymerized enzyme-conjugated antibodies. The enzymatic reaction with chromogenic substrates subsequently leads to the formation of colored precipitates. After counterstaining the nucleus with a nuclear dye, hybridized probe fragments are visualized by light microscopy.

3. Reagents Provided

The Quantum EBV ISH Probe is a ready-to-use ISH probe in a vial made for the use with the Quantum HDx stainer. The vial is equipped with an RFID tag that is read by the Quantum HDx stainer to provide product and lot specific information.

The Quantum EBV ISH Probe is composed of:

- Digoxigenin-labeled oligonucleotides, which target mRNA sequences encoding EBER-1 and EBER-2 regions.

4. Materials Required but Not Provided

- IHC Detection kit
- Positive and negative control specimens
- Microscope slides, positively charged
- Ethanol or reagent alcohol
- Xylene
- Coverslips (22 mm x 22 mm, 24 mm x 60 mm)
- Mounting solution (alcoholic)
- Adequately maintained light microscope

5. Storage and Handling

Store at 2-8°C in an upright position.

Prior to opening the vial, shake down liquid. Return to storage conditions immediately after use. Do not use reagents beyond expiry date indicated on the label. The product is stable until expiry date indicated on the label when handled accordingly.

6. Warnings and Precautions

- Read the instruction for use prior to use!
- Do not use the reagents after the expiry date has been reached!
- This product contains substances (in low concentrations and volumes) that are harmful to health and potentially infectious. Avoid any direct contact with the reagents. Take appropriate protective measures (use disposable gloves, protective glasses, and lab garments)!
- Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
- If reagents come into contact with skin, rinse skin immediately with copious quantities of water!
- A material safety data sheet is available on request for the professional user.
- Do not reuse reagents.
- Avoid cross-contamination of samples as this may lead to erroneous results.

Hazard and precautionary statements:

This product is not classified as hazardous according to Regulation (EC) No. 1272/2008.

7. Limitations

- For research use only.
- For professional use only.
- Specimen staining, especially signal intensity and background staining, is dependent on the handling and processing of the specimen prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other specimens or fluids may produce artefacts or false results. Inconsistent results may result from variations in fixation and embedding methods, as well as from inherent irregularities within the specimen.

8. Interfering Substances

The following fixatives are incompatible with ISH:

- Bouin's fixative
- B5 fixative
- Acidic fixatives (e.g., picric acid)
- Zenker's fixative
- Alcohols (when used alone)
- Mercuric chloride
- Formaldehyde/zinc fixative
- Hollande's fixative
- Non-buffered formalin



9. Preparation of Specimens

- Fixation in 10% neutrally buffered formalin for 24 h at room temperature (18-25°C).
- Use premium quality paraffin.
- Embedding should be carried out at temperatures lower than 65°C.
- Prepare 3-5 µm microtome sections.
- Use positively charged microscope slides.
- Fix for 2-16 h at 50-60°C.

10. Preparatory Treatment of the Device

The product is ready-to-use. No reconstitution, mixing, or dilution is required. Bring probe to room temperature (18-25°C) before use, protect from light. Prior to opening the vial, mix by vortexing and shake down briefly.

11. Assay Procedure

The Quantum EBV ISH Probe is intended to be used on the Quantum HDx stainer in combination with the Quantum detection kits. Please refer to the respective instructions for use for further information.

1. Follow the Quantum HDx stainer for use to setup the reagent for use on the instrument.
2. Load slides, ISH probe and the respective kit onto the Quantum HDx stainer according to the Quantum HDx stainer instruction for use.

Set the pepsin digestion time according to conditions prevalidated by the user.

3. When the staining run is complete, remove the slides from the instrument.
4. Dehydrate with 100% ethanol three times for each 30 sec and in xylene two times for each 30 sec.
5. Air dry samples.
6. Mount with mounting solution and cover with coverslip.
7. Store the slides at room temperature.

12. Interpretation of Results

A positive reactivity in target cells is indicated by cytoplasmic staining. Depending on the detection system that is used, colored precipitates, which can be clearly distinguished from the background, will be observed within the cells targeted by the Quantum EBV ISH Probe.

Please note:

- Visualization of signals should be performed at least at 400- to 630- fold magnification resulting in easily visible signals.
- Do not evaluate areas of necrosis, overlapping nuclei, over-digested nuclei and nuclei with weak signal intensity.
- A negative or unspecific result can be caused by multiple factors (see chapter 15 "Troubleshooting").
- Do not evaluate patient tissue if controls are not as expected
- In order to correctly interpret the results, the user must validate this product prior to use in diagnostic procedures according to national and/or international guidelines.

13. Recommended Quality Control Procedures

In order to monitor correct performance of processed specimens and test reagents, each assay should be accompanied by controls. If the controls fail to demonstrate appropriate staining, results with patient specimens must be considered invalid.

Positive tissue control: A positive tissue control must always be run with every staining procedure performed. This tissue may contain both positive and negative cells and serves as both the positive and negative control tissue. Known positive tissue controls should be utilized for monitoring the correct performance of processed tissues and test reagents, not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate appropriate positive staining, results with the patient specimens must be considered invalid.

Negative tissue control: The same tissue used for the positive tissue control may be used for the negative tissue control.

Negative cells on the slide serve as internal control and have to demonstrate absence of specific staining and can therefore serve as negative tissue control. If these cells demonstrate inappropriate staining, results with the respective specimen must be considered invalid.

14. Disposal

The disposal of reagents must be carried out in accordance with local regulations.

15. Troubleshooting

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all.

Weak signals or no signals at all

Possible cause	Action
No target sequences available	Use appropriate controls
Proteolytic pretreatment not carried out properly	Optimize pepsin incubation time, increase or decrease if necessary
Microscope adjusted wrongly	Adjust correctly

Cross hybridization signals; noisy background

Possible cause	Action
Proteolytic pretreatment too strong	Reduce pepsin incubation time

Tissue morphology degraded

Possible cause	Action
Cell or tissue sample has not been properly fixed	Optimize fixing time and fixative
Proteolytic pretreatment not carried out properly	Optimize pepsin incubation time

Signals fade or merge

Possible cause	Action
An unsuitable mounting solution has been used	Use only the mounting solution provided with the kit or recommended by the instructions for use. Use solutions free of any impurities; do not use coverslip tape

Inconsistent results

Possible cause	Action
Insufficient drying before probe application	Extend air-drying
Variations in tissue fixation and embedding methods	Optimize fixation and embedding methods
Variations in tissue section thickness	Optimize sectioning

Tissue sections wash off the slide

Possible cause	Action
Unsuitable slide coating	Use appropriate (positively charged) slides
Proteolytic pretreatment too strong	Shorten pepsin incubation time
Insufficient drying of the tissue section	Adjust the time to dry the tissues sufficiently before staining
Fixation in formalin that was not properly neutral buffered	Use appropriate neutral buffered formalin of high quality
Tissue thickness	Adjust the thickness of the tissue sections to 3-5 µm



16. Literature

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