# Epstein–Barr virus [EBV] [HHV-4] Real-time PCR Kit

IVD -25°C -15°C

**(For Qualitative Detection)**

REF **8011**



## INTENDED USE

Epstein–Barr virus [HHV4] Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection and quantification EBV specific DNA.

## KIT CONTENTS

| Components | Number of vials | Volume Per vials |
|---|---|---|
| Probe PCR Master Mix | 1 | 250µl |
| EBV Primer Probe Mix [EBV PP mix] | 1 | 65µl |
| Internal control Primer Probe Mix [IC PP Mix] | 1 | 65µl |
| Internal control template [IC template] | 1 | 125µl |
| EBV Positive control [QS1] | 1 | 250ml |
| Water, PCR grade | 1 | 4ml |

## STORAGE

1. The kit is shipped on gel ice. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
2. Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
3. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

## PRODUCT DESCRIPTION

Epstein–Barr virus [HHV4] Real-time PCR Kit constitutes a ready-to-use system for the detection of EBV specific DNA using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of the conserved region of the EBV genome, and for the direct detection of the specific amplicon in FAM channel. In addition, it contains an internal control amplification system to identify possible PCR inhibition and DNA purification efficiency. External positive control is supplied, which can be used as both qualitative and quantitative to determination the amount of viral load.

## SPECIFICITY

EBV primer and probe have been designed for the specific and exclusive in vitro quantification of EBV. The target sequence (EBNA-1 nuclear antigen) is highly conserved and has previously been shown to be a good genetic marker for EBV. The primers and probe sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

## ANALYTICAL SENSITIVITY

The analytical sensitivity is defined as the concentration of DNA molecules (copies/µl) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified EBV specific DNA from 0.001copies to 10copies/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 0.35 copies per micro liter.

## DNA PURIFICATION

If you are using a spin column based sample preparation procedure having washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 3min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the DNA.

## INTERNAL CONTROL TEMPLATE

When performing DNA extraction, it is often advantageous to have an exogenous source of nucleic acid template that is spiked into the lysis buffer. This internal control nucleic acid template is then co-purified with the sample DNA and can be detected as a positive control for the extraction process. Successful co- purification and real-time PCR for the control template also indicates that PCR inhibitors are not present at a high concentration.

The primer and probe present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the internal control template does not interfere with detection of the pathogen target gene even when present at low copy number. The Internal control is detected through the HEX channel and gives a CT value of 23 +/-6.

Add 5µl of the internal control template to each test sample. Do not add directly to test sample. Add after adding lysis buffer to the test sample [sample/lysis buffer mix]. Complete purification according to the manufacturer's protocols.

## DETECTION PROTOCOL

1. Pipette 90µl of PCR grade water into three 1.5ml micro centrifuge tubes and label as QS2 to QS4.
2. Pipette 10µl of Positive control-QS1 into tube QS2.
3. Vortex thoroughly and spin down briefly.
4. Change pipette tip and pipette 10µl from tube QS2 into tube QS3.
5. Vortex thoroughly and spin down briefly.
6. Repeat steps 4 and 5 to complete the dilution series.
7. Use 10µl per reaction.

| Standards | Copies per µl |
|---|---|
| QS-1 | 2500000 |
| QS-2 | 250000 |
| QS-3 | 25000 |
| QS-4 | 2500 |

## DETECTION PROTOCOL

- Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
- Make sure that Positive and Negative control is included in every run.
- Make sure that internal control template is added during DNA purification. If not, pipette 2.5µl of the internal control template directly into the purified DNA.
- Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions.

| Components | Volume per reaction |
|---|---|
| Probe PCR Master Mix | 10µl |
| EBV PP Mix | 2.5µl |
| IC PP Mix | 2.5µl |
| Master Mix Volume | 15µl |
| **Purified DNA** | **10µl** |
| Final reaction volume | 25µl |

**Negative Control setup [NTC]**
Add 10µl of PCR grade water.

**Qualitative Positive Control setup**
Add 10µl of any one of the Positive controls [From QS1 to QS4]

**Quantitative Positive controls setup**
10µl of all Positive controls prepared from QS1 to QS4.

## PROGRAMMING THERMAL CYCLER

| | |
|---|---|
| Sample volume | 25µl |
| Fluorescence Dyes | FAM & HEX |
| Passive reference | None |
| Ramping rate | Default |

**Thermal Profile**

| Cycles | Step | Time | Temp |
|---|---|---|---|
| 1 | Taq enzyme activation / Hold | 15min | 95ºC |
| 45 | Denaturation | 20sec | 95ºC |
| | Annealing/Data collection* | 20sec | 56ºC |
| | Extension | 20sec | 72ºC |

| Data collection/Acquisition | Targets |
|---|---|
| FAM | EBV |
| HEX | Internal control |

## READING THE GRAPH

**Step-1 – Internal control Validation**
Select the test samples alone for the internal control analysis. Select HEX dye and view the graph of internal control amplification. A successful amplification Ct value must be within Ct 21 +/- 10.

This range indicates NO PCR inhibition in the reaction. Any sample value goes beyond Ct 31 indicates that either sample has some issues in the purification or inhibiting PCR reaction.

Internal control will not get amplified in the negative and positive controls. Ignore a late noise HEX amplification graph in the NTC and Positive control wells.

**Step-2 – FAM - Negative and Positive control validation**
Select the NTC and Positive control [Qualitative] or Standards wells [Quantitative], select FAM channel, and view the graph of amplification.

The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The PC or Standards must be amplified as per their copy numbers.

NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage conditions and reaction parameters are as prescribed.

**Step-3 –FAM - Test Sample status**
In FAM channel, select test sample well one by one, analyze the graph/amplification.

## QUALITATIVE INTERPRETATION OF RESULTS

| Test Sample | Negative Control | Positive Control | Internal Control | Interpretation |
|---|---|---|---|---|
| Positive | Negative | Positive | Positive | EBV specific DNA detected |
| Negative | Negative | Positive | Positive | No EBV specific DNA Detected. Sample does not contain detectable amounts of EBV specific DNA. |
| Negative | Negative | Negative | Negative | Experiment fail |
| Positive | Positive | Positive | Positive | Experiment fail |

**Calculating copies or IU per ml**

Input the machine indicated copy number into the following formula

$$\text{Result (copies/ml)} = \frac{\text{Result (copies/µl) x Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

**Note:**

**Elution volume:** must be typed in micro liter format, example 30µl, 60µl or 100µl. **Sample volume:** must be typed in milliliter format, example 0.2ml or 0.5ml

## LIMITATIONS

A false negative result may occur if inadequate numbers of organisms are present in the sample due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

Potential mutations within the target regions of the pathogen's genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect.

As with any diagnostic test, the EBV Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.

## QUALITY CONTROL

In accordance with the Biomolecules in house Quality Management System, each lot of EBV Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality

**Index of symbols**

| | | |
|---|---|---|
| Consult instructions for use | LOT Batch code | Tests per kit |
| IVD For *in vitro* diagnostic use only | Do not re-use | Keep dry |
| 2°C 8°C Temperature limit - 25 °C to - 15 °C | Date of manufacture | Biologial Risks |
| Manufacturer | Use-by date | |

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