

# Epstein-Barr Virus (EBV) Real Time PCR Assay probe/primer mix

## Product Insert

**FOR RESEARCH USE ONLY**

### Introduction:

Denovo Biotechnology's EBV qPCR 20x probe mix is a reliable probe for measuring relative amounts or total copy numbers of EBV by quantitative PCR. The probe consists of forward and reverse primers combined with a FAM/BHQ-labeled probe specific for the BALF5 (DNA polymerase) gene of EBV. This dual-labeled probe is supplied with PCR primers at 20x concentration and may be used with standard real-time PCR reagents.

For absolute copy number calculations, a tube of standards is also included containing the target sequence at  $10^{12}$  copies per ml. These standards produce linear results at dilutions of  $10^8$  to  $10^2$  copies per reaction.

### Kit Components:

1. EBV Dual Labeled probe/primer mix at 20x concentration (18uM each forward and reverse primers, 5uM dual-labeled probe) Volume 250 ul, enough for 250 20-ul reactions or 100 50-ul reactions.  
**Store at -20° C**
2. EBV standard at  $10^{12}$  copies per ml  
**Store at -20° C**

### Materials Required but not supplied:

1. Taq polymerase, Taq buffer, dNTP mix. These are often supplied together as a complete mixture for real-time PCR, often in 2x concentration.
2. Nuclease-free H<sub>2</sub>O
3. DNA samples isolated by the procedure of your choice, dissolved in nuclease-free H<sub>2</sub>O
4. Real-time PCR detection system capable of detecting emission from FAM.

### Procedure:

- Isolate DNA samples using the procedure of your choice, making serial dilutions in nuclease-free H<sub>2</sub>O.
- Add 1/20 of the final reaction volume of EBV probe/primer to your chosen real-time PCR mix (Taq polymerase, dNTPs, buffer, nuclease-free water). For example, use 1 ul per 20 ul reaction and 2.5 ul per 50 ul reaction.
- To minimize pipetting errors, it is recommended to combine a sufficient quantity of all the above reagents for all of the standards and samples to be assayed, distribute the appropriate volume into each reaction tube, and then add the template DNA.
- To determine absolute copy numbers, make serial 1/10 dilutions of the Standard Tube ( $10^{12}$  copies per ml). Standards produce linear results at a range of  $10^8$  to  $10^2$  copies per reaction.

To determine the number of copies in a sample, a standard curve is constructed as follows:

1. Construct a graph of the Ct values for the standards on the Y axis and the log of the copy number per reaction on the X axis.
2. Use the Ct values of the Unknown Sample to determine the concentration from the curve. Alternatively, enter the data into a calculator or a software program and calculate the sample concentrations by linear regression.

Most qPCR software can perform the above calculations automatically.

