Human Herpesvirus 6A & 6B Real Time PCR Assay probe/primer mix

Product Insert

FOR RESEARCH USE ONLY

Catalog No.: H6K243

Introduction:

Denovo Biotechnology's HHV-6A & 6B qPCR 20x probe mixes are reliable probes for measuring relative amounts or total copy numbers of HHV-6A or HHV-6B by quantitative PCR. The probes consist of forward and reverse primers combined with a FAM/BHQ-labeled probe which bind within the Immediate-Early region of the genome. These probes can bind specifically with their particular variant A or B) due to splicing differences in the IE region. Each dual-labeled probe mix is at 20x concentration and may be used with standard real-time PCR reagents.

For absolute copy number calculations, two tubes of standards are also included containing the target sequences for probes HHV6A or HHV6B 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. HHV-6A 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.
2. HHV-6A standard at 10^{12} copies per ml
3. HHV-6B 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.
4. HHV-6B standard at 10^{12} copies per ml

   Store all components 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 H2O
3. DNA samples isolated by the procedure of your choice, dissolved in nuclease-free H2O
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 H2O.
- Add 1/20 of the final reaction volume of either HHV-6A or HHV-6B 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.