

Quantification of Human Papillomavirus 6

E6 protein

For general laboratory and research use only

Standard kit

150 tests

Introduction to Human Papillomavirus 6

Papillomaviruses are a diverse group of DNA-based viruses that infect the skin and mucous membranes of humans and a variety of animals. Although more than 100 different human papillomavirus (HPV) types have been characterized HPV-6, HPV 11, HPV 16 and HPV 18 are the most clinically relevant.

Genital or anal warts (condylomata acuminata or venereal warts) are the most easily recognised sign of genital HPV infection. Although a wide variety of HPV types can cause genital warts, types 6 and 11 account for about 90% of all cases. HPV types 16 and 18 are called "high-risk" types because they can cause cervical cancer, as well as anal cancer, vulvar cancer, head and neck cancers, and penile cancer. HPV-induced cancers often have viral sequences integrated into the cellular DNA. Some of the HPV "early" genes, such as E6 and E7, are known to act as oncogenes that promote tumor growth and malignant transformation.

Specificity

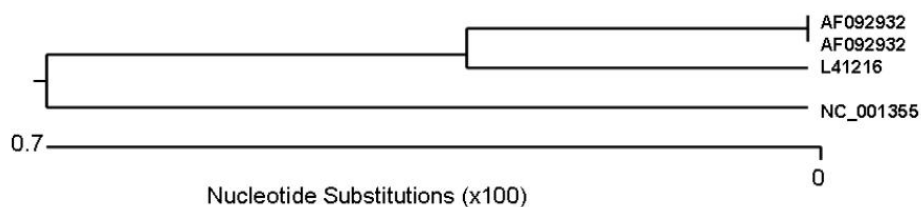
The PrimerDesign™ genesig Kit for Human Papillomavirus 6 (HPV6) Genomes is designed for the in vitro quantification of HPV6 genomes. The kit is designed to have the broadest detection profile possible whilst remaining specific to the HPV6 genome.

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.

The primers have 100% homology with all reference sequences included in the phylogenetic tree below and therefore have a very broadest quantification profile. However, due to the instability of viral genomes, it is not possible to guarantee quantification of all clinical isolates.

If you require further information, or have a specific question about the detection profile of this kit then please send an e.mail to enquiries@primerdesign.co.uk and our bioinformatics team will answer your question.

Table 1 Accession numbers for detected HPV-6 isolates



Kit Contents

- HPV6 specific primer/probe mix (150 reactions **BROWN**)
FAM labeled, BHQ quenched
- HPV6 positive control template (for Standard curve **RED**)
- RNase/DNase free water

Reagents and equipment to be supplied by the user

Real-Time PCR Instrument

DNA extraction kit

This kit is designed to work well with all processes that yield high quality DNA with minimal PCR inhibitors.

Mastermix or Mastermix components

This kit is designed to work well with all commercially available Mastermixes. However, we recommend the use of PrimerDesign 2x Precision™ MasterMix.

Pipettors and Tips

Vortex and centrifuge

Thin walled 1.5 ml PCR reaction tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20°C on arrival. PrimerDesign does not recommend using the kit after the expiry date stated on the pack. Once the lyophilized components have been re-suspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity (An internal PCR control is supplied to test for non specific PCR inhibitors). Always run at least one negative control with the samples. To prepare a negative-control, replace the template RNA sample with RNase/DNase free water.

Dynamic range of test

Under optimal PCR conditions PrimerDesign HPV6 detection kits have very high priming efficiencies of $>95\%$ and can detect less than 100 copies of target template.

Notices and disclaimers

During the warranty period PrimerDesign genesig detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. "Black Hole Quencher", "BHQ", "CAL Fluor", "Quasar" and "Pulsar" are registered trademarks of Biosearch Technologies, Inc., Novato, CA. This technology is protected by U.S. and World-wide patents either issued or in application and is licensed and sold under agreement with Biosearch Technologies, Inc. These products are sold exclusively for R&D use by the purchaser. They may not be used for human or veterinary in vitro diagnostic (IVD) applications and they may not be re-sold, distributed or re-packaged without express written authorization from Biosearch Technologies Inc. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applied Biosystems Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc. and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation. The purchase of Biosearch Technologies products does not, either expressly or by implication, provide a license to use this or other patented technology. Licensing information can be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404 or the Licensing Department at Roche Molecular Systems Inc., 1145 Atlantic Avenue, Alameda, CA 94501."

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The purchase of the PrimerDesign™ reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-La Roche Inc.

Principles of the test

Real-time PCR

A HPV6 specific primer and probe mix is provided and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the HPV6 DNA/cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of real-time PCR platforms.

Positive control

For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template. This can be used to generate a standard curve of HPV6 copy number / CT value. Alternatively the positive control can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target HPV6 gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To confirm the absence of contamination, a negative control reaction should be included every time the kit is used. For this reaction, the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. If a positive result is obtained the results should be ignored and the test samples repeated. Possible sources of contamination should first be explored and removed.

Carry-over prevention using UNG (optional)

Carry over contamination between PCR reactions can be prevented by including uracil-N-glycosylase (UNG) in the reaction mix. Some commercial mastermix preparations contain UNG or alternatively it can be added as a separate component. UNG can only prevent carry over from PCR reactions that include deoxyuridine triphosphate (dUTP) in the original PCR reaction. PrimerDesign recommend the application of 0.2U UNG per assay with a 15 minute incubation step at 37°C prior to amplification. The heat-labile UNG is then inactivated during the Taq polymerase activation step (95°C for 10 minutes).

Bench side Protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.
2. **Reconstitute the kit components according to the table below.**
To ensure complete resuspension, vortex each tube thoroughly.

Component	Volume
Pre-PCR transparent envelope	
HPV6 Primer/Probe mix (BROWN)	165 μ l
Post-PCR heat-sealed foil	
Positive Control Template (RED) *	500 μ l

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

Real-time PCR detection

- Prepare a reaction mix according to the tables below:**
Include sufficient reactions for the standard curve wells (6 samples in duplicate) and also the negative control.

HPV6 detection mix

Component	Volume
2 x Precision™ MasterMix	10 µl
HPV6 Primer/Probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	15 µl

- Pipette 15µl of this mix into each well according to your real-time PCR experimental plate set up.**
- Prepare sample DNA templates for each of your samples (suggested concentration 5ng/µl) in RNAse/DNAse free water.**
If the concentration of DNA is not known, then dilute your DNA sample reactions 1:20 (10µl of sample DNA and 190µl of water).
- Pipette 5µl of diluted DNA template into each well, according to your experimental plate set up.**
For negative control wells use 5µl of RNAse/DNAse free water. The final volume in each well is 20µl.
- Preparation of standard curve dilution series.**
 - 1) Pipette 900µl of RNAse/DNAse free water into 5 tubes and label 2-6
 - 2) Pipette 100µl of Positive Control Template (RED) into tube 2
 - 3) Vortex thoroughly
 - 4) Change pipette tip and pipette 100µl from tube 2 into tube 3
 - 5) Vortex thoroughly

Repeat steps 4 and 5 to complete the dilution series

Standard Curve	Copy Number
Tube 1 Positive control (RED)	2×10^5 per μl
Tube 2	2×10^4 per μl
Tube 3	2×10^3 per μl
Tube 4	2×10^2 per μl
Tube 5	20 per μl
Tube 6	2 per μl

6. **Pipette 5 μl of standard template into each well, according to your experimental plate set up.**
 The final volume in each well is 20 μl .

Amplification Protocol

Amplification conditions using PrimerDesign 2X Precision™ MasterMix.

	Step	Time	Temp
	UNG treatment (if required) **	15 mins	37 °C
	Enzyme activation (if required) ***	10 mins	95 °C
50 Cycles	Denaturation	10s	95 °C
	DATA COLLECTION *	60s	60 °C

* Fluorogenic data for the control DNA should be collected during this step through the FAM and VIC channels

** Required if your Mastermix includes UNG to prevent PCR carryover contamination

*** Not all Mastermixes require this enzyme activation step. Follow the manufacturers instructions for your Mastermix.