Primerdesign

Z-Path-High Risk HPV

High-Risk Human Papillomavirus

Kit version: 1

genesig® Multiplex Screening Kit

100 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Product Description

This genesig® Multiplex qPCR detection kit targets the most common high-risk human papillomavirus genotypes.

Papillomaviruses are a diverse group of DNA viruses that infect epithelial tissues of the skin and mucous membranes of humans and a variety of animals. More than 100 different human papillomavirus (HPV) genotypes have been characterized, and they are generally classified as low-risk or high-risk according to their oncogenic potential.

HPV is one of the most common sexually transmitted infections worldwide. Most infections are asymptomatic and clear through immune responses. When symptoms do occur, low-risk HPV types, with genotypes 6 and 11 accounting for about 90% of all cases, may cause benign lesions such as genital warts. However, persistent infection with high-risk HPV types can lead to precancerous cellular changes and malignant disease, particularly cervical cancer, but also anal cancer, vulvar cancer, head and neck cancers, and penile cancer. High-risk genotypes account for over 90% of cervical adenocarcinomas worldwide, with HPV16 and HPV18 being most common, emphasizing the importance of high-risk HPV testing.

This kit facilitates screening for high-risk HPV genotypes within a single reaction. The following genotypes can be detected: HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66 and HPV68.

The multiplex test uses four fluorescent channels to confirm presence of HPV16 (ROX), HPV18 (VIC) and other high-risk HPV genotypes (FAM), alongside an endogenous control (Cy5). HPV16 and HPV18 together account for 70% of positive cases in clinical practice, highlighting the importance of detection and differentiation of both genotypes specifically. All other high-risk genotypes make up the remaining 30% of clinical positives and are grouped together in the FAM channel. This configuration allows a partial genotyping result with differentiation of the two most common high-risk genotypes.

Specificity

The kit is designed for the in vitro detection of high-risk HPV genomes and to have a broad detection profile.

The kit contains individual primers and probes specific to each of the following high-risk HPV genotypes: HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68. The primers and probes are designed to exclude detection of low-risk and other clinically irrelevant HPV genotypes.

The dynamics of genetic variation means that new sequence information may become available after the initial design. If you require further information or have a specific question about the detection profile of this kit, then please send an e-mail to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

<table>
<tr><th>Quantity</th><th>Component</th><th>Tube</th><th>Cap Colour</th></tr>
<tr><td>1</td><td>High risk HPV primer/probe mix (100 reactions)
<table><tr><th>Target</th><th>Fluorophore</th></tr><tr><td>HPV16</td><td>ROX</td></tr><tr><td>HPV18</td><td>VIC</td></tr><tr><td>HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, HPV68</td><td>FAM</td></tr><tr><td>Human ACTB endogenous control</td><td>Cy5</td></tr></table></td><td>  </td><td>BROWN</td></tr>
<tr><td>1</td><td>High risk HPV positive control template</td><td>  </td><td>RED</td></tr>
<tr><td>2</td><td>oasig® lyophilised 2X qPCR Master Mix (50 reactions per glass vial)</td><td>  </td><td>SILVER</td></tr>
<tr><td>2</td><td>oasig® resuspension buffer
For resuspension of the lyophilised Master Mix</td><td>  </td><td>BLUE</td></tr>
<tr><td>1</td><td>RNase/DNase free water
For resuspension of the primer/probe mix</td><td>  </td><td>WHITE</td></tr>
<tr><td>1</td><td>Template preparation buffer
For resuspension of the positive control template</td><td>  </td><td>YELLOW</td></tr>
</table>

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

DNA Extraction kit

This kit is recommended for use with genesig® Easy DNA/RNA extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high-quality nucleic acid with minimal PCR inhibitors.

Pipettors and filter tips

Vortex and centrifuge

1.5 ml microtubes or qPCR plates

Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and DNA integrity.

Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template.

Principles of the test

Real-time PCR

Individual primers and probes designed for each genotype (ROX, VIC, FAM) and endogenous control (CY5) have been combined into a single reaction and these can be detected through the different fluorescent channels as described in the kit contents above.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target DNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled 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 qPCR platforms.

Positive control

For a positive control, the kit includes a single positive control tube that contains templates for HPV16, HPV18 and a representative for the other high-risk HPV genotypes detected by this kit. This will give a signal in each channel used for HPV detection (ROX, VIC and FAM). 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 probe for the respective target 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 controls do 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 validate any positive findings, 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. It is also known as a No Template Control or NTC.

Endogenous control

To confirm extraction of a valid biological sample, the multiplex primer/probe mix supplied contains primers and probe designed to detect a human endogenous gene. Detection of the endogenous control is through the Cy5 channel. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

Resuspension protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting is 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/probe mix or template is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the high risk HPV primer/probe mix in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension allow primer/probe mix to rehydrate for 10 minutes at room temperature. Vortex the tube thoroughly, followed by pipetting up and down 10 times. Failure to mix well can produce poor kit performance.

Component - resuspend in water	Volume
Pre-PCR pack	
High risk HPV primer/probe mix (**BROWN**)	110 µl

3. **Resuspend the positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
High risk HPV Positive Control Template (**RED**) *	500 µl

* 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.

4. **Resuspend the lyophilised oasig 2X qPCR Master Mix in oasig resuspension buffer, according to the table below:**

Component - resuspend in oasig resuspension buffer	Volume
Lyophilised oasig Master Mix	525 µl

DNA extraction

Complete the DNA extraction according to the manufacturer's recommended protocol.

qPCR detection protocol

1. **For each DNA sample prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

Component	Volume
oasig® lyophilised 2X qPCR Master Mix	10 µl
High risk HPV primer/probe mix (**BROWN**)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15 µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Pipette 5 µl of DNA template into each well according to your experimental plate set up.**
   For negative control (NTC) wells use 5 µl of RNase/DNase free water. The final volume in each well is 20 µl.

4. **Pipette 5µl of positive control template into the positive control wells.**

qPCR amplification protocol

Recommended amplification conditions when using oasig® lyophilised 2X qPCR Master Mix:

	Step	Time	Temp
	Enzyme activation	2 min	95 ℃
Cycling x50	Denaturation	10 s	95 ℃
	DATA COLLECTION *	60 s	60 ℃

* Fluorogenic data should be collected during this step through the FAM, VIC, ROX and Cy5 channels.

As the HPV16 assay produces amplification through the ROX channel, please ensure ROX normalisation is switched off if using a qPCR instrument that recommends ROX as a passive reference dye.

Interpretation of results

Positive Control

The positive control well should give an amplification plot through the FAM, VIC, and ROX channels. The positive control signals indicate that the kit is working correctly.

The number of copies of template packaged in the positive control varies for each channel and therefore the Cq value will be different. The expected Cq values are listed below:

Channel	Expected Cq value
ROX	21-25
VIC	17-21
FAM	24-29

Failure of the positive control to amplify within these Cq ranges and satisfy these quality control criteria is a strong indication that the experiment has been compromised. If a negative result is obtained in either the ROX, VIC or FAM channel, then the test results are invalid and must be repeated.

Negative control

The negative control should give a flat line (flat amplification plots) through all channels. Signals in the negative control indicate cross-contamination during plate setup.

Endogenous control

The signal obtained from the endogenous control primer and probe set in the Cy5 channel will vary according to the amount of biological material present in a given sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.

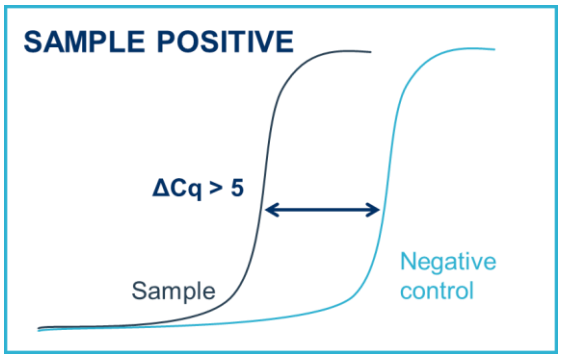
If the endogenous control fails to amplify, this indicates an issue with the sample quality or quantity and the test for this sample has failed. In these situations, ensure that a sample from human origin was used, and try increasing the concentration of the DNA sample that is added to the reaction, or checking the DNA extraction protocol for any user errors during preparation and repeat the DNA extraction. Please note, poor extractions can be caused by overloading the DNA extraction with too much starting material.

Sample data

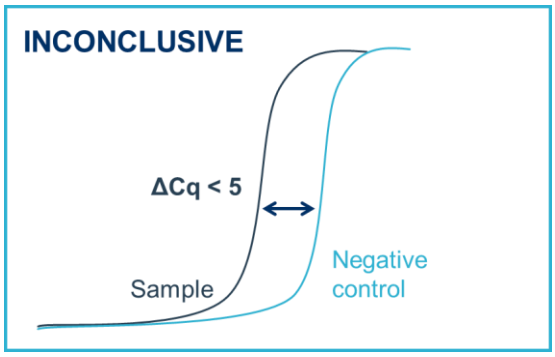
Presence of HPV is detected in the channels indicated in the kit contents section. A positive signal in the ROX channel indicates a positive test for HPV16. A positive signal in the VIC channel indicates a positive test for HPV18, whereas a positive signal in the FAM channel indicates positive tests for HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66 or HPV68. Mixed infections are relatively common, with reported frequencies of up to 25% depending on the clinical setting. That means that samples may give positive signals through multiple channels such as FAM and VIC e.g. a sample containing HPV18 (VIC channel) and HPV68 (FAM channel). Mixed infections can also lead to unusually shaped curves where more than one amplification takes place in the same channel e.g. a sample where both HPV58 and HPV66 are present and amplify, and produce fluorescence in the FAM channel.

Target (ROX/VIC/FAM)	Endogenous control (Cy5)	Positive Control	Negative Control	Interpretation
ROX +	**+ / -**	**+**	**-**	**HPV16 POSITIVE RESULT**
VIC +	**+ / -**	**+**	**-**	**HPV18 POSITIVE RESULT**
FAM +	**+ / -**	**+**	**-**	**OTHER HIGH-RISK GENOTYPE POSITIVE RESULT**
-	**+**	**+**	**-**	**NEGATIVE RESULT**
+ / -	**+ / -**	**+**	**≤35**	**EXPERIMENT FAILED** Due to test contamination
+ / -	**+ / -**	**+**	**>35**	*****
-	**-**	**+**	**-**	**SAMPLE PREPARATION FAILED**
+ / -	**+ / -**	**-**	**+ / -**	**EXPERIMENT FAILED**

*Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primer Design Ltd 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.

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