YOUSEQ

HIGH RISK HPV MULTIPLEX qPCR TEST KIT FOR STEPONEPLUS MACHINES

CAT NO.: YSL-qPX-EC-HiRi.HPV.SO-100

100 reactions
With Endogenous control and lyophilised MasterMix

VERSION 2.0

For Research Use Only

YOUSEQ

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INTENDED USE

The YouSeq® High Risk HPV Multiplex qPCR Kit for StepOnePlus Machines is a qualitative *in-vitro* Polymerase Chain Reaction (PCR) assay that utilises hydrolysis probe detection technology for the detection of high-risk Human Papillomavirus (HPV) DNA in cervical cells collected in liquid cytology media. The assay is intended to detect 14 high risk HPV genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and to partially genotype 16 and 18 from the other 12 high risk genotypes. It is designed to be used by trained users in a suitable molecular biology laboratory environment. The primers/probes are shown in silico to have 100% specificity to the targets.

KIT CONTENTS

	Cap Colour	Volume
High Risk HPV specific primer/probe set HPV 16 (VIC/HEX probe) HPV 18 (NED probe) HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 & 68 (FAM probe) Endogenous control (ROX probe)		110 µl
Positive Control Template		500 µl*
Lyophilised Tetra 2X qPCR MasterMix		1 .1 ml*
MasterMix resuspension buffer		1.5 ml
Template resuspension buffer		1.5 ml
RNase/DNase free water		1.5 ml

* Supplied lyophilised and requires resuspension, see resuspension step below for instructions

RESUSPENSION STEP

Resuspend the designated kit contents with the correct reagents as per the table below. Spin or gently tap the vial to ensure all the contents is at the bottom before opening. After adding the resuspension reagent, pulse vortex the vial to ensure it is mixed well.

	Reagent	Volume
Positive Control Template	Template resuspension buffer	500 µl
Lyophilised Tetra 2X qPCR MasterMix	MasterMix resuspension buffer	1.1 ml

MATERIALS REQUIRED BUT NOT PROVIDED

DNA Extraction Kit – This qPCR test kit will work well with high quality DNA derived from any extraction kit with minimal PCR inhibitors present.
Pipettes, micro-centrifuge tubes and general laboratory equipment.

RECOMMENDED INSTRUMENT

This qPCR kit will work on any instrument StepOnePlus instrument that detects VIC/HEC, ROX, FAM and NED.

If you are not using a StepOne Plus machine, we recommend using our alternative kit below:

Product Description	Catalogue Number
High Risk HPV Multiplex qPCR Test Kit	YS-qPX-EC-HiRi.HPV-100

qPCR BENCH SIDE PROTOCOL

Clean and decontaminate all work surfaces, pipettes, and other equipment prior to use to remove potentially contaminating nucleic acids.

REACTION SET UP

Combine the following reagents to create a final test reaction:

Component	Volume
Tetra 2X qPCR MasterMix	10 µl
Primer/probe mix	1 µl
Extracted Sample DNA	9 µl
Final Volume	20 µl

NEGATIVE CONTROL

For a negative control reaction, repeat the reaction set up above replacing the sample DNA with DNase/RNase free water.

Please note: Make sure to seal the sample and negative control wells before proceeding to the positive control step.

POSITIVE CONTROL

For a positive control reaction, repeat the reaction set up above but replace the extracted sample DNA with 9 µL of the resuspended positive control template supplied with the kit.

qPCR AMPLIFICATION PROTOCOL

Run the following PCR protocol:

Please note: If using a qPCR machine that uses ROX as a passive reference, then the passive reference must be turned off or set to "none" indicating no passive reference.

	Temperature	Time
Hot Start	95°C	3 minutes
6 cycles	95°C	15 seconds
	60°C	60 seconds
39 cycles	95°C	15 seconds
	60°C*	60 seconds

*Data collection only in final 39 cycles for all target channels – VIC/HEC, ROX, FAM and NED

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INTERPRETATION OF RESULTS

Setting the correct threshold

We recommend that all thresholds should be placed at 15% of the final end point fluorescence of the positive control template for the relevant target.

Positive Control

Firstly, check the positive control performance. It should amplify in a Cq range of approximately 12.5+/-2 for all targets. If the Cq range is not achieved, this would be a failed test and should be repeated.

Negative Control

In ideal circumstances, the negative control well should deliver a flat line – negative result. However, it is not uncommon for background laboratory contamination to cause a very late signal. If this signal is ≥5 Cq values later than any of the HPV targets, then it can be considered negative, and the result is viable.
If the negative is <5 Cq later than the sample result, then the result is inconclusive, and the test should be repeated after potential sources of contamination have been removed.

Positive Samples

Samples that are positive for the target will cross the threshold set previously and deliver a defined "sigmoidal" amplification plot.

Endogenous control

If your sample delivers a strong positive result for the target, then the endogenous control is not required for data interpretation and can be ignored.
If your samples deliver a negative result, then the endogenous control is useful to interpret the result. The Cq value from the endogenous control will vary according to the amount of DNA in your sample. A late signal (Cq>22) indicates that only a small amount of host derived DNA was present in your sample. You may wish to repeat sample collection and then repeat the test in order to confirm the negative result.

Results interpretation:

	qPCR Signal				
HPV 16 (VIC/HEX probe)	+	-	-	-	-
HPV 18 (NED probe)	-	+	-	-	-
HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 & 68 (FAM probe)	-	-	+	-	-
Endogenous control (ROX probe)	+/-	+/-	+/-	+	-
Result	**Positive result** HPV 16 specific DNA detected.	**Positive result.** HPV 18 specific DNA detected.	**Positive result.** HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 or 68 DNA detected.	**Negative result.**	**Failed test.** Repeat required

Coinfection

On the rare occasion that a sample contains more than one HPV subtype, positive signals in multiple channels will be observed.

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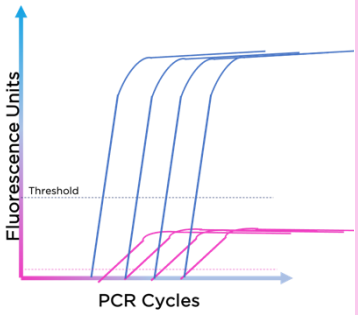
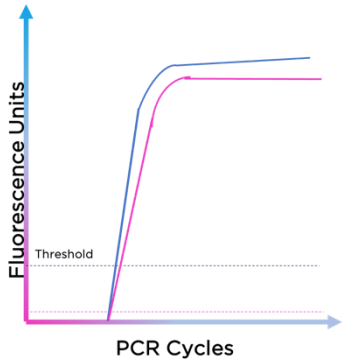
MULTIPLEX TROUBLESHOOTING

	Trace	What can you see?	Cause	Action
1		One assay with greater end point fluorescence than another	Some fluorophores are brighter than others. Also, certain instruments detect different fluorophores with higher/lower efficiency	Analyse each channel individually so the Y-axis is correct for each fluorophore. or Analyse on logarithmic scale instead of linear scale
2		Amplification/ unusual Cq value in unexpected fluorescent channel. Cq value/curve shape very similar to adjacent fluorescent channel	'Bleed through' or 'cross talk' between channels. Amplification from one fluorescent channel has been mistakenly identified in its adjacent channel (e.g., FAM identified as HEX)	Ensure manufacturer recommends the dye combination used in this kit. Recalibrate qPCR instrument.

PRODUCT SPECIFICATIONS

Storing your kit

Store at -20C from arrival. The qPCR kits shelf life is outlines as an expiry date on the pouch label.
Once you have prepared the positive control it can be stored frozen.

Use good quality DNA

Poor quality input nucleic acid is the biggest cause of test failure. The kit will work well with any source of good quality DNA. Good quality is defined as DNA with high integrity (not degraded) and with low levels of inhibitors present.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use. It should not be used for diagnosis of disease unless specifically approved by the regulatory authorities in the country of use.

Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of High Risk HPV Multiplex qPCR Test Kit for StepOnePlus Machines is tested against predetermined specifications to ensure consistent product quality.

Technical Assistance

For customer support, please contact:

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Not available in all countries.