HELINI

Human Papillomavirus [HPV] 14 high risk Viruses genotyping Real-time PCR Kit

[HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68]

Instructions for use

For use with: Agilent, Bio-Rad, Roche Lightcycler-96, Roche-Z480/Cobas-480, Applied Bio systems [ABI], Thermo-Piko-Real, Rotor gene 5/6plex, Alta-96, Cepheid Real time PCR machines.

Cat. No: 8023- 25 / 50 / 100 tests

CE IVD

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HELINI Biomolecules, Chennai, INDIA

Intended Use

The HELINI HPV 14 high-risk virus Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection and genotyping HPV 14 high risk virus specific DNA.

Kit components

No. of reactions	25
Probe PCR Master Mix 10µl/reaction	8 x 250µl
HPV 16/18/Endogenous Primer Probe Mix [HPV 16/18/Endo PP mix] - 5µl/reaction	125µl
HPV 31/35 Primer Probe Mix [HPV 31/35 PP mix] - 5µl/reaction	125µl
HPV 33/45 Primer Probe Mix [HPV 33/45 PP mix] - 5µl/reaction	125µl
HPV 39/52 Primer Probe Mix [HPV 39/52 PP mix] - 5µl/reaction	125µl
HPV 51/58 Primer Probe Mix [HPV 51/58 PP mix] - 5µl/reaction	125µl
HPV 56/59 Primer Probe Mix [HPV 56/59 PP mix] - 5µl/reaction	125µl
HPV 66/68 Primer Probe Mix [HPV 66/68 PP mix] - 5µl/reaction	125µl
Positive control Mix - 10µl/reaction	250µl
Instruction manual	

Storage

- The kit is shipped on gel ice. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
- Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
- If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

Material and instruments required

- Real-time PCR instrument having FAM & HEX & Cy5 channels
- Automatic Nucleic acid extraction system or spin column based purification kit for the purification of nucleic acids
- Desktop centrifuge having 13000rpm or above with a rotor for 1.5/2 ml reaction tubes
- Centrifuge with a rotor for PCR strips/tubes and 96 well plates
- Optical cap qPCR tubes or strips or 96 wells
- Micro Pipettes (variables)
- Micro Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

[Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.]

Product Use Limitations

- All reagents may exclusively be used in molecular diagnosis.
- The product is to be used by personnel specially instructed and trained in Molecular diagnosis.
- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens and kit components.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Use separated and segregated working areas for sample preparation, reaction setup and amplification/detection activities.
- The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.

- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.

Technical Assistance

For technical assistance and more information, please contact;

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Introduction

HELINI Human Papillomavirus 14 high risk viruses Real-time PCR Kit constitute a ready-to-use system for the detection and genotyping of HPV 14 high risk viruses using Real-time polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification conserved region in the HPV genome, and for the direct detection of the specific amplicon in fluorescence channels FAM and HEX. In addition, it contains a human gene amplification system [endogenous-human gene] to identify biological status of the test sample, possible PCR inhibition and DNA purification. External positive control mix is supplied to assist run.

Specificity

HPV 14 high risk viruses Primer and Probe have been designed for the specific and exclusive *in vitro* quantification of high risk HPV viruses. **[HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68]** The target sequence (E6/E7 gene) is highly conserved and has previously been shown to be a good genetic marker for HPV. 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.

Dynamic range of test

Under optimal PCR conditions, the kit have very high priming efficiencies of >95% and can detect 10 copies and less of target template.

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 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.

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.

Endogenous control

Human gene provided as endogenous control. It amplifies a single copy human gene from the test samples. No amplification indicates that either sample has some issues in the purification or inhibiting PCR reaction or Sample not suitable for qPCR. Ask for fresh test sample.

Detection Protocol

Things to do before starting

- Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
- Make sure that Positive and Negative control is included in every run.
- Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions.

Detection Mix – Sample validation

Components	16/18/ Endo	31/35	33/45	39/52	51/58	56/59	66/68
Probe PCR Master Mix	10µl	10µl	10µl	10µl	10µl	10µl	10µl
PP Mix	5µl	5µl	5µl	5µl	5µl	5µl	5µl
Purified DNA sample	**10µl**	**10µl**	**10µl**	**10µl**	**10µl**	**10µl**	**10µl**
Total	25µl	25µl	25µl	25µl	25µl	25µl	25µl

Centrifuge PCR vials briefly before placing into thermal cycler. *[Note: There should not be any bubbles in the reaction mix. Bubbles interfere with fluorescence detection.]*

Negative Control setup [NTC]
Add 10µl of PCR grade water.

Qualitative Positive control
Add 10µl of Positive control mix

Programming Thermal cycler

Sample volume	25µl
Fluorescence Dyes	FAM & HEX & Cy5
Passive reference	None
Ramping rate	Default

	Step	Time	Temp
	Taq enzyme activation	15min	95ºC
40 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec**	60ºC
	Extension	20sec	72ºC

** Some qPCR machines may require minimum 30sec for data collection; in that case, set to 30sec, this will not affect the performance.

Channels

FAM	HEX	Cy5
16	18	Endogenous control
35	31	
45	33	
52	39	
58	51	
59	56	
68	66	

Reading the graph:

Step-1 – Endogenous control Validation

Select only test samples for endogenous control analysis. Select FAM and view the graph of endogenous control amplification. A successful amplification Ct value must be 24 +/- 9 and justify;

- Test sample collected properly [required quantity of human cells are present in the sample] and retains its biological property.
- NO PCR inhibition in the qPCR reaction.
- DNA purification is successful.

Step-2 – FAM - Negative and Positive control validation

Select the NTC and Positive control, select FAM channel, and view the graph of amplification.

The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The PC must be amplified.

NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage conditions and reaction parameters are as prescribed.

Step-3 – Test Sample status

Select first FAM channel, view the graph of test samples one by one. And then select HEX channel, view the graph of test samples. Keep NTC always when analyzing test sample amplification.

Limitations

Good laboratory practice is essential for proper performance of this assay. Strict compliance with the instructions for use is required for optimal results.

Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.

A false negative result may occur if inadequate numbers of organisms are present in the sample due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

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Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

Potential mutations within the target regions of the pathogen's genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect.

As with any diagnostic test, the HELINI HPV 14 high virus genotyping Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.

Quality Control

In accordance with the HELINI Biomolecules in house Quality Management System, each lot of HELINI HPV 14 high risk virus Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

Manufactured by

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Explanations of symbols

In vitro diagnostic medical device

Catalogue number

Pack size – number of tests

Manufacturer