

GeneProof Human Papillomavirus (HPV) PCR Kit



In vitro diagnostic medical device

The kit has been manufactured according to the EC Directive 98/79/EC as an *in vitro* diagnostic medical device and it has been designed for professional use in specialized clinical and research laboratories.

KIT CONTENT

REF	ISEX Version		
	HPVS/ISEX/025	HPVS/ISEX/050	HPVS/ISEX/100
	25 rxn	50 rxn	100 rxn
MasterMix			
HPV	1x375 µl	2x375 µl	4x375 µl
Positive Control			
HPV	1x200 µl	1x200 µl	2x200 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kit could be transported at temperature below -20 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). The components are stable for a maximum of 5 repeated freezing / thawing cycles after the first use of a particular vial. The component must be used before the expiry date or 14 days after the first use of a particular vial (whichever comes first).

TECHNICAL SPECIFICATION

Target Sequence	E1/E2 genes
Specificity	Human Papillomavirus high-risk types 16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82, 97 with differentiation of 16, 18 and 45 types
Sensitivity (LoD)	reaches up to 881.5 IU/ml for HPV 16 reaches up to 936.7 IU/ml for HPV 18
Validated specimens	cervical, penis and vaginal swab, LBC
External Quality Assessment	regularly tested by the QCMD and Instand e.V. External Quality Assessment Panels
Regulatory status	CE IVD

Quality management system is certified in compliance with the requirements of the standard ISO 13485 ed. 2:2016.

METHOD PRINCIPLES

The PCR kit is designed for the detection of 24 high-risk types of Human Papillomavirus (HPV) and typing of HPV 16, 18, 45 by the real-time Polymerase Chain Reaction (PCR) method. The HPV detection consists in amplification of a specific conservative DNA sequence in the area of *E1/E2* genes and in measurement of fluorescence increase. The kit enables detection of following high-risk types HPV: 16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82, 97. The high-risk HPV types presence is indicated by the FAM, fluorescence growth. The kit enables simultaneously type HPV 16, 18 and 45. The HPV16 presence is indicated in the Cy5 fluorescence channel, HPV 18 in the TexRed fluorescence channel and HPV 45 in the Cy5.5 fluorescence channel. The primers for *GAPDH* gene are present in the reaction mix for the DNA extraction quality control and possible PCR inhibition control. Amplification of *GAPDH* gene is indicated in the HEX fluorescence channel. The detection kit takes advantage of the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity. Ready to Use MasterMix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR by amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

ISEX version

Internal Standard is detected from the sample. This PCR kit version enables both PCR inhibition control and nucleic acid purification process efficiency control.

MICROBIOLOGICAL DNA DIAGNOSTIC TECHNOLOGY

/ SAMPLE

positive sample contains
viral DNA
DNA for human *GAPDH*



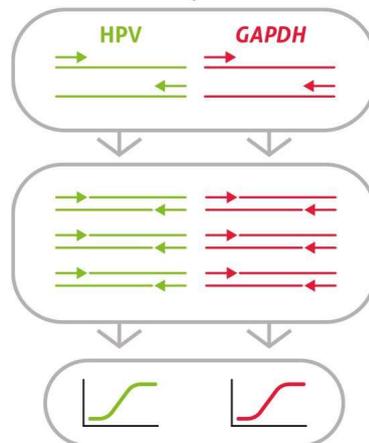
/ DNA ISOLATION

after the isolation the extracted sample DNA is added into Ready to Use MasterMix and the tube is inserted into the real-time device



/ PCR AMPLIFICATION

during PCR, viral DNA is amplified from one primer pair and control human DNA amplified from the other primer pair



/ EVALUATION

POSITIVE SAMPLE

- exponential fluorescence growth of the FAM, Cy5, Texas Red or Cy5.5 fluorophore is evident if the target viral DNA is present in the sample

QUALITY CONTROL FOR THE COMPLETE DIAGNOSTIC PROCESS

- exponential growth of the HEX fluorophore fluorescence, as a result of the control human DNA amplification, controls the following:
 - sample quality –sample DNA (and therefore also the viral RNA) was not degraded
 - DNA extraction quality –sample DNA was extracted with sufficient efficiency
 - PCR amplification quality –sample DNA was efficiently amplified, no PCR inhibition

USER MANUAL

SAMPLING AND SAMPLE STORAGE

It is possible to use cervical, penis and vaginal swab and LBC for HPV detection. Using self-testing devices (Evalyn Brush) is also possible. All specimens can be stored up to 48 h in temperature range from +2 °C to +8 °C. For long term storage it's necessary to keep samples frozen at the temperature -20 ± 5 °C.

NUCLEIC ACID PURIFICATION

Nucleic acid isolation should be performed by isolation kits available at the market according to protocols for the particular clinical material isolation. The manufacturer recommends the following isolation kits:

croBEE NA16 Nucleic Acid Extraction System
DNA extraction MCHF16+ cartridge code 110
GeneProof PathogenFree DNA Isolation Kit
Siemens VERSANT® kPCR Molecular System

PCR SETUP

1. Add 15 µl of MasterMix into PCR tubes.

2. Add 5 µl of the isolated nucleic acid sample or 5 µl of Positive Control into the individual PCR tubes. The final reaction mix volume will be 20 µl. **WARNING!** Reaction volume has to be set to 40 µl. It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. The customer has to use his own negative control in the form of negative clinical material in each test.

3. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following PCR profile. Be very careful when handling the Positive Control or the clinical material, incorrect handling could result in contamination and the consequent impairment of the kit components or the MasterMix! The manufacturer is not responsible for the kit impairment due to incorrect handling.

AMPLIFICATION PROFILE

Step	Temperature	Time	Data Collection	Cycles
Hold	42 °C	15 min		1
Hold	95 °C	10 min		1
PCR	95 °C	5 s		45
	60 °C	40 s	FAM+HEX+Cy5+TexRed+Cy5.5	
	72 °C	20 s		
Hold	10 °C	1 min		1

INSTRUMENTS

GeneProof Human Papillomavirus (HPV) PCR Kit is designed for use with real-time devices from various manufacturers:

	HPV HR	IC	HPV 16	HPV 18	HPV 45
croBEE Real-Time PCR System	FAM	HEX	Cy5	TexRed	Cy5.5
Applied Biosystems 7500 Real-Time PCR System	FAM	JOE	Cy5	TexRed	-
CFX Connect™/CFX96™/Dx Real-Time PCR Detection System	FAM	HEX	Cy5	TexRed	Quasar 705
LineGene 9600/9600 Plus	FAM	HEX	Cy5	TexRed	Cy5.5
Rotor-Gene Q	FAM	JOE	Cy5	TexRed	Quasar 705
SLAN® Real-Time PCR System	FAM	HEX	Cy5	-	-

Required channels: FAM, HEX, Cy5, TexRed, Cy5.5

GeneProof diagnostic kits are continually verified with various types of devices. Current list is available at www.geneproof.com or request the list at support@geneproof.com.



CLINICAL SAMPLE ANALYSIS EVALUATION

Interpretation

FAM	Cy5	TexRed	Cy5.5	HEX	
+	-	-	-	+/-	<i>High risk HPV</i>
+	+	-	-	+/-	<i>HPV 16*</i>
+	-	+	-	+/-	<i>HPV 18*</i>
+	-	-	+	+/-	<i>HPV 45*</i>
+	+	+	-	+/-	<i>HPV 16 and HPV 18*</i>
+	+	+	+	+/-	<i>HPV 16, HPV 18 and HPV 45*</i>
+	+	-	+	+/-	<i>HPV 16 and HPV 45*</i>
+	-	+	+	+/-	<i>HPV 18 and HPV 45*</i>
-	-	-	-	+	<i>NEGATIVE</i>
-	-	-	-	-	<i>INVALID RESULT</i>

* The FAM channel should always be positive. Positivity in FAM channel represents presence of at least one of 24 high risk HPV types in the sample. In the case of positivity of other channels except HEX which allow differentiation of HPV 16, 18 and 45, it is possible coinfection with other high risk HPV types.

In case of highly positive HPV, low signal can appear in channel Cy5 (CFX Connect™/CFX96™/DX Real-Time PCR Detection System)

WARNING

A single valid Instruction for use for a specific kit is included in the package or to be requested for the particular lot from the manufacturer. The kit should be disposed of after use according to the current legal regulations considering the fact that the kit does not contain any dangerous, infectious or toxic components that would be subject to special safety regulations, and the packaging materials are made of paper and polypropylene. If you have any questions please contact our Customer Service.

Customer care and technical support

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