



Multiplex REAL-TIME PCR Detection Kits

HPV 6,11

REF R1-P321-23/9INT
 R1-P321-S3/9INT

HPV 16,18

REF R1-P320-23/9INT
 R1-P320-S3/9INT

General information

Intended use:

HPV 6,11 and **HPV 16,18 Multiplex REAL-TIME PCR Detection Kits** are intended for detection and typing of two most oncogenic and persistent high-risk human papilloma virus types (HPV 16, HPV 18) and two low-oncogenic risk human papilloma virus types (HPV 6, HPV 11) by method of multiplex Real-Time PCR.

Kit formats:

HPV 6,11 is intended for detection and typing of types 6 and 11 human papilloma virus.

HPV 16,18 is intended for detection and typing of types 16 and 18 human papilloma virus.

Method:

Multiplex Real-Time PCR, qualitative analysis.

Samples:

Epithelial cell swabs from urethra, cervical canal, uterine neck.

DNA extraction:

The DNA-Technology's **PREP-GS** and **PREP-NA** and **PREP-RAPID** (not applicable to male urethral swabs) extraction kits are recommended.

Features:

Multiplex analysis gives the opportunity of simultaneous detection and differentiation of several HPV types in the same tube.

PCR-Mix contains an internal control (IC). IC is intended for PCR quality assurance.

We also recommend including in assay the negative control (C-) which is not supplied but very helpful for contamination control purposes. Use deionized water or sterile buffered saline instead of sample, starting from extraction step.

Devices:

The automatic analysis for **HPV 6,11** and **HPV 16,18 Multiplex REAL-TIME PCR Detection Kits** is available on "DNA-Technology" made DTlite², DTprime² REAL-TIME Thermal Cyclers; the latest version of the software is available for download at <http://www.dna-technology.com/software>.

Time of analysis (including sample preparation procedure):

from 2.5 hours.

The number of tests:

96 (including one positive control and one negative control in each run).

Kit contents:

Reagent		Organoleptic parameters	Quantity	
1.	Paraffin sealed PCR-mix	Colorless transparent liquid under white wax layer	20 µL in each	12 8-tubes strips or 96 tubes
2.	Taq-polymerase solution	Colorless transparent liquid	500 µL	2 tubes
3.	Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	2 tubes
4.	Positive control	Colorless transparent liquid	150 µL	1 tube
Associated accessories: Strip's caps ³				12 8-caps

¹ - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments

² - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments

³ - for detection kit packaged in strips **REF** R1-P321-S3/9INT, **REF** R1-P320-S3/9INT

Dye label detection channels

Fam	Hex	Rox	Cy5	Cy5.5
HPV6	IC	-	HPV11	-
HPV18	IC	-	HPV16	-

Procedure

1 PCR amplification



The reagents and tubes should be kept away from direct sun light!



Strictly observe the completeness of the strips and caps to them. Do not use the caps to the strips of the other kits!

1.1 Mark the required number of the tubes with paraffin sealed PCR-mix considering samples, negative control (C-) and positive control (C+).

Example: to test 2 samples, mark 2 tubes for samples, one for "C-", one for "C+". The resulting number of tubes is 4.

1.2 Vortex the Taq-polymerase solution thoroughly (3-5 s), then spin briefly (1-3 s).

1.3 Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.

1.4 Add one drop (~20 µL) of mineral oil into each tube.

1.5 Vortex the tubes with samples, "C-" and "C+" for 3-5 s and spin down drops for 1-3 s.



1. In case of using **PREP-GS extraction kit**. After vortexing centrifuge the tubes with the DNA preparation at 16 000 x g for one minute at room temperature (from 18 °C to 25 °C) to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 s in a vortex-mixer.

Relative centrifugal force (RCF or g) depends on rotation frequency and rotor radius (Annex A). To establish if your centrifuge meets the requirements apply to the exploitation manual for centrifuge.

2. Open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next DNA sample to prevent contamination. Use filter tips. Close tubes/strips tightly.

1.6 Add 5.0 µL of the DNA sample into corresponding PCR-tubes. Avoid paraffin layer break. Do not add DNA into the "C-", "C+" tubes.

1.7 Add 5.0 µL of negative control sample (C-), which passed whole DNA extraction procedure into corresponding tube. Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

1.8 Spin tubes/strips for 1-3 s to collect drops.

1.9 Set the tubes/strips to real-time PCR thermal cycler.

1.10 Launch the RealTime_PCR application in "Device operation" mode. Upload corresponding ini file before the first run. Add test in subsequent runs. Specify the number and identifiers of samples. Define position of tubes/strips in software interface according to position they were set in thermal unit (p. 1.9). Run PCR.

2 Data collection and data analysis

Registration of the PCR results is held in automatic mode. Interpretation of the PCR results should be performed according to the Table 1.

Table 1 Interpretation of PCR results

Detection channel		Result	Result interpretation
Fam, Cy5	Hex		
Analyzed samples			
Cp is specified (in the one or two detection channels)	Is not considered	+	DNA of one or two HPV types is present (HPV16 and/or HPV18, HPV6 and/or HPV11)
Cp is not specified (in the both detection channels)	Cp is specified	-	DNA of 6/11 or 16/18 HPV types is not present
Cp is not specified (in the both detection channels)	Cp is not specified (in the both detection channels)	n/a	Unreliable result
Positive control sample			
Cp is specified (in the both detection channels)	Cp is specified	+	Positive result The results are valid
Negative control sample			
Cp is not specified (in the both detection channels)	Cp is specified	"-"	Negative result The results are valid

Storage, shipping and handling requirements

All kit components should be stored at the temperatures from 2 °C to 8 °C during the storage period.



Paraffin-sealed PCR-mix should be stored at the temperatures from 2 °C to 8 °C and out of light during the storage period.

The kit can be transported in thermal containers with icepacks by all types of roofed transport at temperatures corresponding to the storage conditions of the kit components over the transportation. Transportation is allowed in thermal containers with icepacks by all types of covered transport at temperatures from 2 °C to 25 °C inside the container, but for no longer than 5 days.

Shelf-life – 12 months if all the conditions of transportation, storage and operation are met.

Contact our customer service department regarding quality issues with the kit:













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Key to symbols

	Temperature limitation		Consult instructions for use		Catalogue number
	Expiration date		Manufacturer		Batch code
	Date of manufacture		Number of tests		Do not expose to sunlight
	Caution		Not sterile		Single use

Nomogram and formula for calculation of relative centrifugal force (RCF) in the speed of rotation (RPM) depending of the rotor diameter

