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For research use only

INSTRUCTION FOR USE
HPV 6,11 Multiplex REAL-TIME PCR Kit

REF **R1-P321-23/9EU**
R1-P321-S3/9EU

General information

Intended use:
HPV 6,11 Multiplex REAL-TIME PCR Kit is intended for detection and typing of two low-oncogenic risk human papilloma virus types (HPV 6, HPV 11) by method of multiplex Real-Time PCR.

Method:
Multiplex Real-Time PCR, qualitative analysis.

Samples:
Epithelial cell scrapes 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 Multiplex REAL-TIME PCR Kit** is available on "DNA-Technology" made DTlite[1] and DTprime[2] REAL-TIME Thermal Cyclers; the latest version of the software is available for download at https://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 waxy white fraction	20 µL in each	12 8-tubes strips or 96 tubes
2. Taq-polymerase solution	Colorless transparent liquid	500 µL in each	2 tubes
3. Mineral oil	Colorless transparent viscous oily liquid	1.0 mL in each	2 tubes
4. Positive control[3]	Colorless transparent liquid	150 µL	1 tube
Associated accessories:			
Strip's caps [4]			12 8-caps

Dye label detection channels

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

[1] - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments
[2] - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments
[3] marking as C+ is allowed
[4] - for detection kit packaged in strips REF R1-P321-S3/9EU

Procedure

1 PCR amplification

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

When using strips, 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 DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 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 on a vortex mixer.
Relative centrifugal force RCF(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 strip to prevent contamination. Use filter tips. Close tubes/strips tightly.

1.6 Add 5.0 µL of the DNA sample into corresponding PCR-tubes. Open the tube, add DNA sample, then close the tube before proceeding to the next DNA sample to prevent contamination. Use filter tips. 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 Vortex tubes for 1–3 s to collect drops.
1.9 Set the tubes/strips to real-time PCR thermal cycler.
1.10 Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (1.9) and run PCR.

2 Data collection and data analysis

Registration of the PCR results are 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 HPV6 and/or HPV11 is present
Cp is not specified (in the both detection channels)	Cp is specified	-	DNA of HPV6 and HPV11 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

[1] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[2] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

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 excessive temperature and light can be detrimental to product performance.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions.

It is allowed to transport the kit in thermobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2°C to 8°C immediately on receipt.

Shelf life – 12 months since the date of production in compliance with all transportation, storage and operation conditions.

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

8 (800) 200.75.15 (toll-free call for Russia)

+7 (495) 640.16.93 (chargeable call for CIS and foreign countries).

E-mail: hotline@dna-technology.ru

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Key to symbols

RUO	For research use only		Consult instructions for use	REF	Catalogue number
	Temperature limit		Manufacturer	LOT	Batch code
	Use-by date		Contains sufficient for <n> tests		Keep away from sunlight
	Date of manufacture	NON STERILE	Non-sterile		Caution

Annex A

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