



HSV1, HSV2, CMV Multiplex REAL-TIME PCR Detection Kit

Package S

REF R1-P210-23/9EU
 R1-P210-S3/9EU

General information

Intended use:

HSV1, HSV2, CMV Multiplex REAL-TIME PCR Detection Kit is intended for detection of Herpes simplex virus 1 (HSV1), Herpes simplex virus 2 (HSV2) and Cytomegalovirus (CMV) DNA in human biological samples by method of multiplex Real-Time PCR. Current modification of the method allows simultaneous detection and differentiation of HSV1, HSV2 and CMV DNA in the same tube.

HSV1, HSV2, CMV Multiplex REAL-TIME PCR Detection Kit can be used in scientific research practice.

Method:

Multiplex Real-Time PCR, qualitative analysis.

Samples:

Urina; epithelial cell scrapes from urethra, cervical canal, posterior vaginal vault.

DNA extraction:

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

Features:

Multiplex analysis gives the opportunity of simultaneous detection and differentiation of HSV1, HSV2 and CMV DNA 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 **HSV1, HSV2, CMV Multiplex REAL-TIME PCR Detection Kit** is available on "DNA-Technology" made DTlite¹ and DTprime² REAL-TIME Thermal Cyclers; the latest version of the software is available for download at <https://www.dna-technology.com/software>.

Time of analysis (excluding sample preparation procedure):
 from 2 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. MAX 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-P210-S3/9EU.

Dye label detection channels

Fam	Hex	Rox	Cy5	Cy5.5
Herpes simplex virus 2 (HSV2)	IC	Cytomegalovirus (CMV)	Herpes simplex virus 1 (HSV1)	-

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. If you need to test 5 samples, mark 7 tubes (one for each sample, one for "C-", one for "C+").

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

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

- 1.4** Add one drop (~20 µL) of mineral oil into each tube. Close tubes/strips.

- 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** and **PREP-GS PLUS** extraction kits. 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 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. In case of using **PREP-MB RAPID Extraction Kit**. The DNA samples must stand in a magnetic rack while adding DNA. 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.

3. 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 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 operating software for DT instrument¹. Add corresponding test², 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 (see 1.9) and run PCR.

2 Data collection and data analysis

Registration and interpretation of the PCR results are held in automatic mode.



Cp on the Fam, Rox, Cy5 channels less than 24 indicates high initial DNA concentrations of corresponding pathogen that may cause false-negative results for low-presented pathogen. In this case repeating of PCR amplification using DNA-Technology **HSV 1,2 REAL-TIME PCR Detection Kit** and **CMV REAL-TIME PCR Detection Kit** is recommended.

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

HSV1, HSV2, CMV Multiplex REAL-TIME PCR Detection Kit 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 has to be transported in thermoboxes 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 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 limit		Consult instructions for use		Catalogue number
	Use-by date		Manufacturer		Batch code
	Date of manufacture		Contains sufficient for <n> tests		Keep away from sunlight
	Caution		Non-sterile		Do not reuse

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

