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USER MANUAL
REAL-TIME PCR DETECTION KIT
General information

Intended use:
The Real-Time PCR Detection Kits are in vitro DNA tests, which are intended for the specific identification of various microorganisms in human biological samples.
Method:
Real-time polymerase chain reaction; qualitative detection.
DNA extraction:
The "DNA-Technology" PREP-NA, PREP-GS and PREP-RAPID DNA extraction Kits are recommended. Some types of the samples must be pretreated (refer to the corresponding user manuals and full version of the Real-Time PCR Detection Kit user manual).

⚠ Use only PREP-NA Kit for DNA extraction from plasma. Use only PREP-NA and PREP-GS Kits for DNA extraction from biopsy samples, sputum, bronchoalveolar lavage, gastric fluid and faeces.
We do not recommend PREP-RAPID DNA Extraction Kit for DNA extraction from male urogenital scrapes.
Features:
PCR-Mix contains an internal control (IC). IC is intended for PCR quality and sufficiency of DNA assurance.
Positive control plasmid ("C+") supplied with the kit is intended for specific PCR assessment.
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 DNA-Technology Real-Time PCR Detection Kits is available on "DNA-Technology" made DT-322, DTlite[1], DTprime[2], DT-96; software version is not lower than 7.3.4.0; the latest version of the software is available for download at http://www.dna-technology.ru/eng/support/
or Bio-Rad Laboratories iCycler iQ thermal cyclers.

⚠ Please enquire DNA-Technology company's representative about compatibility of third-party Real-time instruments.
Overall time needed to perform the analysis (excluding sample preparation procedure):
from 1.5 h.
Number of tests:
48/96

Kit content

Reagent	Quantity	
	48 tests	96 tests
Paraffin sealed PCR-mix	48 tubes or 6*8-tube-strips (20 µL in each tube)	96 tubes or 12*8-tube-strips (20 µL in each tube)
Taq-polymerase solution	1 tube (total volume 480 µL)	2 tubes (480 µL in each tube)
Mineral oil	1 tube (total volume 960 µL)	2 tubes (960 µL in each tube)
Positive control	1 tube (total volume 75 µL)	1 tube (total volume 150 µL)

Detection channels

Fam	Hex	Rox	Cy5	Cy5.5
Specific product	IC	-	-	-

Storage and handling requirements

All components of the Kits must be stored at from 2 °C to 8 °C during the storage period.
Note. The storage of PCR-mix at temperatures from 18 °C to 25 °C in a dark place is allowed during the storage period.

[1] - 4S1, 4S2, 5S2, 6S1, 6S2 models
[2] - 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 models

Transportation can be held by all types of roofed transport at temperatures between 2 °C and 8 °C over the transportation.
Shelf life – 12 months since the date of Quality Control Department approval in compliance with all transportation, storage and operation conditions.

Contact our customer service by quality issues of DNA-Technology Real-Time PCR Detection Kits:
Phone:+7 (800) 200-75-15,
Phone/Fax: +7(495) 640-17-71.
E-mail: hotline@dna-technology.ru, www.dna-technology.ru.
Address: 117587, Moscow, Varshavskoye sh., 125g building 6, DNA Technology.

Samples

Microorganism	Sample
Bordetella pertussis	Scrapes from posterior pharyngeal wall
Candida albicans	Scrapes from urethra, cervix, or posterolateral vaginal wall
Chlamydophila pneumoniae	Sputum, bronchoalveolar lavage, scrapes and washouts from nasal and oral pharynx
Chlamydia trachomatis	Scrapes from urethra, cervix, or posterolateral vaginal wall
Corynebacterium diphtheriae - toxigenic strains	Scrapes from fibrin filmoral pharynx, larynx and other mucous membranes, lesion fluid
Cytomegalovirus (CMV)	Saliva, urine, scrapes from urethra, cervix, or posterolateral vaginal wall, peripheral blood mononuclear cells
Epstein–Barr virus (EBV)	
Gardnerella vaginalis	Scrapes from urethra, cervix, or posterolateral vaginal wall
Helicobacter pylori	Biopsy samples, gastric fluid and faeces
Herpes symplex virus 1,2 (HSV-1,2)	Peripheral lymphocytes, saliva, urine, scrapes from urethra, cervix, or posterolateral vaginal wall
Human herpesvirus 6 (HHV6)	Peripheral lymphocytes, spinal fluid, saliva, urine etc.
Human herpesvirus 8 (HHV8)	Peripheral lymphocytes, sperm, prostate fluid, biopsy samples
Human papillomavirus 6 (HPV6)	Scrapes from urethra, cervix, nasal and oral pharynx, biopsy samples etc.
Human papillomavirus 11 (HPV11)	
Human papillomavirus 16 (HPV16)	
Human papillomavirus 18 (HPV18)	
Human Parvovirus B19	Blood plasma, saliva, biopsy samples from heart valves, amniotic fluid, autopsy samples
Legionella pneumophila	Sputum, bronchoalveolar lavage
Listeria monocytogenes	Spinal fluid, mucosal scrapes, amniotic fluid, meconium, biopsy samples
M. tuberculosis – M. bovis	Sputum, bronchoalveolar lavage, the contents of tuberculoma
Mycoplasma genitalium	Scrapes from urethra, cervix, or posterolateral vaginal wall
Mycoplasma hominis	
Mycoplasma pneumoniae	Sputum, bronchoalveolar lavage, scrapes and washouts from nasal and oropharynx
Neisseria gonorrhoeae	Scrapes from urethra, cervix, or posterolateral vaginal wall
Streptococcus agalactiae	Scrapes from vagina, rectum, nasal pharynx, urine, spinal fluid, blood, autopsy samples
Streptococcus pneumoniae	Sputum, bronchoalveolar lavage, scrapes and washouts from nasal and oral pharynx
Streptococcus pyogenes	Scrapes from urethra, cervix, posterolateral vaginal wall, lesions and mucosa of the amygdales,posterior pharyngeal wall
Toxoplasma gondii	Spinal fluid, biopsy samples, etc
Trichomonas vaginalis	Scrapes from urethra, cervix, or posterolateral vaginal wall
Ureaplasma parvum	
Ureaplasma urealyticum	
U. urealyticum + U. parvum	
Varicella zoster virus (VZV)	Lesion and mucosa scrape

Procedure

1 PCR amplification

1.1 Mark tubes with paraffin sealed PCR-mix for test samples, negative control ("C-") and positive control ("C+"). **Example**. If you need to test 5 samples, mark 5 tubes for samples, 1 tube for "C-" and 1 tube for "C+". Total number of tubes - 7.

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

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. Close tubes tightly.

1.5 Add 5,0 µL of DNA sample into corresponding PCR-tubes. Avoid paraffin layer break. 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.

1.6 Add 5,0 µL of "C-" which passed whole DNA extraction procedure and "C+" into corresponding tubes. Avoid paraffin layer break.

1.7 Spin tubes briefly (1-3 sec).

1.8 Set the tubes to Real-time PCR instrument.

1.9 For DT-322, DTlite, DTprime and DT-96 thermal cyclers:
Launch RealTime_PCR software and choose the Device handling mode. Create and save new test if you do this test for the first time. In subsequent runs add the saved test to the protocol, specify the number and ID's of the samples, specify the position of the tubes in the thermal unit (1.8) and run PCR (see table 1).
For iCycler iQ thermal cyclers:
Switch on the thermal and optical units of the device and let it warm up for 30 min. Launch iCycler (or Bio-Rad iQ5) software. Create and save new protocol if you do this protocol for the first time. In subsequent runs add the saved protocol, configure the plate (create the file with the data on samples ID's and position) and run PCR considering the total PCR reaction volume equal to 35 µL (see tables 2, 3).

⚠ When working with *Gardnerella vaginalis* REAL-TIME PCR Detection Kit the PCR can be ran according to the common for all Kits protocol. In this case, consider the value of the Ct (Cp) on Fam channel. The samples considered positive if Ct (Cp) value is less or equal to 35.

2 Data collection and data analysis

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

⚠ Real-Time PCR Detection Kits are designed to obtain maximum performance and information capacity when using with real-time thermal cyclers but as alternative the electrophoretic detection can be performed (see table 4 and user manual supplied with electrophoretic detection kit).

Table 1

The PCR program for DT-322, DTlite, DTprime and DT-96 thermal cyclers

Step	Temperature, ºC	Min.	Sec.	Number of cycles		Optical measurement	Type of the step
				For all kits except *G. vaginalis* REAL-TIME PCR Detection Kit	For *G. vaginalis* REAL-TIME PCR Detection Kit		
1	80.0	0	30	1	1		Cycle
	94.0	1	30				
2	94.0	0	30	5	5		Cycle
	64.0	0	15			v	
3	94.0	0	10	45	35		Cycle
	64.0	0	15			v	
4	94.0	0	5	1	1		Cycle
5	10.0	…	…	…	…		Holding

Table 2

The PCR program for iCycler iQ5 thermal cyclers (with persistent well factor)

Cycle	Repeats		Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
	For all kits except *G. vaginalis* REAL-TIME PCR Detection Kit	For *G. vaginalis* REAL-TIME PCR Detection Kit				
1	1	1				
			1	1 min	80.0	
			2	1 min 30 sec	94.0	
2	5	5				
			1	30 sec	94.0	
			2	45 sec	64.0	
3	45	35				
			1	10 sec	94.0	
			2	45 sec	64.0	Real Time
4	…	…	…	…	10.0	Storage

Table 3

The PCR program for iCycler iQ thermal cyclers (with dynamic well factor)

Cycle	Repeats		Step	Dwell time	Setpoint, ºC	PCR/Melt Data Acquisition
	For all kits except *G. vaginalis* REAL-TIME PCR Detection Kit	For *G. vaginalis* REAL-TIME PCR Detection Kit				
dynamicwf.tmo program						
1	1	1				
			1	1 min	80.0	
			2	1 min 30 sec	94.0	
2	5	5				
			1	30 sec	94.0	
			2	45 sec	64.0	
3	2	2				
			1	30 sec	80.0	Real Time
PCR program						
4	45	35				
			1	10 sec	94.0	
			2	45 sec	64.0	Real Time
5	…	…	…	…	10.0	Storage

Table 4

The PCR product length

Microorganism	PCR product length (nucleotide pairs)	IC (nucleotide pairs)
Bordetella pertussis	419	100
Candida albicans	310	560
Chlamydophila pneumoniae	308	100
Chlamydia trachomatis	321	100
Corynebacterium diphtheriae - toxigenic strains	251	560
Cytomegalovirus (CMV)	280	560
Epstein–Barr virus (EBV)	185	100
Gardnerella vaginalis	445	100
Helicobacter pylori	348	100
Herpes symplex virus 1,2 (HSV-1,2)	261	560
Human herpesvirus 6 (HHV6)	277	560
Human herpesvirus 8 (HHV8)	293	560
Human papillomavirus 6 (HPV6)	244	100
Human papillomavirus 11 (HPV11)	244	100
Human papillomavirus 16 (HPV16)	337	560
Human papillomavirus 18 (HPV18)	417	100
Human Parvovirus B19	274	100
Legionella pneumophila	383	100
Listeria monocytogenes	67[3]	100
M. tuberculosis – M. bovis	330	100
Mycoplasma genitalium	203	560
Mycoplasma hominis	310	560
Mycoplasma pneumoniae	210	100
Neisseria gonorrhoeae	329	100
Streptococcus agalactiae	357	100
Streptococcus pneumoniae	327	100
Streptococcus pyogenes	455	100
Toxoplasma gondii	187	560
Trichomonas vaginalis	218	560
Ureaplasma parvum	383	100
Ureaplasma urealyticum	206	560
U. urealyticum + U. parvum	532	100
Varicella zoster virus (VZV)	269	100

[3] - the PCR products for *Listeria monocytogenes* can not be detected in standard 1.5% agarose gel.