117587, Moscow, Varshavskoye sh., 125g, building 6
Phone/Fax +7 (495) 640-17-71
Customer service department: +7 (800) 200-75-15
(toll-free within Russia)
E-mail: hotline@dna-technology.ru,
www.dna-technology.ru

USER MANUAL
CONVENTIONAL PCR DETECTION KIT
General information

Intended use:
The DNA-Technology Conventional PCR Detection Kit is designed for microbial DNA detection in biological samples by polymerase chain reaction (PCR) with gel electrophoresis end-point detection.
Method:
End-point (conventional) PCR, qualitative analysis
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 for DNA extraction kit and full version of the user manual for PCR Detection Kit).

⚠ 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.
Devices:
The DNA-Technology made four-block thermocycler "Tercyc"; power supply (e.g. DNA-Technology made "Elf"); electrophoresis chamber; gel documentation system.

⚠ Please enquire DNA-Technology company's representative about compatibility of third-party instruments.

Overall time needed to perform the analysis (excluding sample preparation procedure):
from 1.5 h.

Number of tests:
50/100

Content

Reagent	Quantity			
	for 50 tests		for 100 tests	
• Paraffin sealed PCR-mix[1]	20 µL	50 tubes	20 µL	100 tubes
• Taq–polymerase solution	500 µL	1 tube	500 µL	2 tubes
• Mineral oil	1,0 ml	1 tube	1,0 ml	2 tubes
• Positive control	75 µL	1 tube	150 µL	1 tube

Storage and handling requirements

PCR kit must be stored at temperatures between 2 °C and 8 °C and out of light during the storage period. The excessive temperature and light can be detrimental to product performance.

⚠ Storage of paraffin sealed tubes with PCR mix in dark place at temperatures between 18 °C and 25 °C is allowed within the whole shelf life period.

Transportation can be held by all types of roofed transport at temperatures between 2 °C and 8 °C over the transportation.

Expiry date – 9 months since the date of Quality Control Department approval in compliance with all transportation, storage and operation conditions.

Contact our customer service department regarding issues of quality of the Conventional PCR detection Kit:
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

[1] - conventional PCR detection Kits are manufactured in 0,5 and 0,2 ml tubes depending on their names.

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
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 simplex virus 1,2 (HSV-1, 2)	Peripheral lymphocytes, saliva, urine, scrapes from urethra, cervix, or posterolateral vaginal wall
Type 6 Human herpes virus (HHV-6)	Peripheral lymphocytes, spinal fluid, saliva, urine etc.
Type 8 Human herpes virus (HHV-8)	Peripheral lymphocytes, sperm, prostate fluid, biopsy samples
Human Papillomavirus types 6 and 11 (HPV 6, 11)	Scrapes from urethra, cervix, rectum, nasal and oral pharynx, biopsy samples etc.
Human papillomavirus 16 (HPV16)	
Human papillomavirus 18 (HPV18)	
Human Papillomavirus types 16, 31, 33, 35, 35H, 52, 58, 67 (HPV 16, 31, 33, 35, 35H, 52, 58, 67)	Scrapes from urethra, cervix, or posterolateral vaginal wall
Human Papillomavirus types 18, 45, 39, 59 (HPV 18, 45, 39, 59)	
Human Papillomavirus types 51 and 26 (HPV 51, 26)	
Mycoplasma genitalium	
Mycoplasma hominis	
Neisseria gonorrhoeae	
Trichomonas vaginalis	
Ureaplasma parvum	
Ureaplasma urealyticum	
Ureaplasma urealyticum+ Ureaplasma parvum	
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
Varicella zoster virus (VZV)	Lesion and mucosa scrape
Vibrio cholerae toxicogenous strains	Bacterial cell suspension

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 all tubes in PCR cycler and carry out PCR under conditions showed in tables 1-4 considering 35 µL reaction mix volume. Using Tercyc cycler you need to choose «Precision active regulation» regulation algorithm. Compliance of PCR conditions with reagent kits is shown in Table 5.

2. Detection and DNA PCR cycling results interpretation

2.1. Upon finishing, PCR results are analyzed with horizontal gel electrophoresis.
(See user's guide to the reagent kit for PCR products detection with the gel electrophoresis method).

2.2. The PCR product is seen in UV light (wavelength 254 nm or 310 nm) as a glowing bright orange DNA band.

2.3. There are two variants possible when using the DNA PCR detection kit with an internal control in case of positive sample:
1) DNA band at the positive control sample level (Table 5);
2) two DNA bands one of which is located at the positive control level and the other corresponds to the DNA Internal control

2.4. In case of absence of specific DNA in a sample (negative sample) only one band corresponding with the internal control is visible.

2.5. An empty gel lane (both internal control and specific fragment band are missing) suggests that the polymerase chain reaction has failed. That might be caused by inhibitor presence in the DNA preparation obtained from clinical material, wrong execution of the analysis protocol, PCR temperature inobservance etc. In that case either repeated PCR of a DNA preparation or clinical material re-sampling is required.

2.6. If a DNA PCR reagent kit is used without an internal control in case of positive sample, a DNA band at the level of positive control sample is visible (Table 5); a lane in the gel is empty if there is no specific DNA in the sample (negative sample).

Table 1

Thermal cycling program 1

№ Block	Thermocyclers with active temperature adjustment			Thermocyclers without active temperature adjustment			Cycles number
	Temperature, °C	Time		Temperature, °C	Time		
		min	sec		min	sec	
1	94,0	1	30	94,0	1	30	1
2	94,0 64,0 72,0	0 0 0	20 5 5	94,0 64,0 72,0	0 0 0	50 50 50	5
3	94,0 64,0 72,0	0 0 0	5 5 5	94,0 64,0 72,0	0 0 0	50 50 50	40
4	10,0	…	…	10,0	…	…	storage

Table 2

Thermal cycling program 2

№ Block	Thermocyclers with active temperature adjustment			Thermocyclers without active temperature adjustment			Cycles number
	Temperature, °C	Time		Temperature, °C	Time		
		min	sec		min	sec	
1	94,0	1	30	94,0	1	30	1
2	94,0 67,0 72,0	0 0 0	20 5 5	94,0 67,0 72,0	0 0 0	50 50 50	5
3	94,0 67,0 72,0	0 0 0	5 5 5	94,0 67,0 72,0	0 0 0	50 50 50	40
4	10,0	…	…	10,0	…	…	storage

Table 3

Thermal cycling program 3

№ Block	Thermocyclers with active temperature adjustment			Thermocyclers without active temperature adjustment			Cycles number
	Temperature, °C	Time		Temperature, °C	Time		
		min	sec		min	sec	
1	94,0	1	30	94,0	1	30	1
2	94,0 67,0 72,0	0 0 0	20 5 5	94,0 67,0 72,0	0 0 0	50 50 50	5
3	94,0 67,0 72,0	0 0 0	5 5 5	94,0 67,0 72,0	0 0 0	50 50 50	30
4	10,0	…	…	10,0	…	…	storage

Table 4

Thermal cycling program 4

№ Block	Thermocyclers with active temperature adjustment			Thermocyclers without active temperature adjustment			Cycles number
	Temperature, °C	Time		Temperature, °C	Time		
		min	sec		min	sec	
1	94,0	1	30	94,0	1	30	1
2	94,0 62,0 70,0	0 0 0	20 10 10	94,0 62,0 70,0	0 0 0	50 50 50	5
3	94,0 64,0 70,0	0 0 0	5 5 5	94,0 64,0 70,0	0 0 0	50 50 50	40
4	10,0	…	…	10,0	…	…	storage

Table 5

Lengths of PCR Products

PCR product	PCR product length, bp	Internal control length, bp
Thermal cycling program1		
Chlamydia trachomatis	415	900
Chlamydophila pneumoniae	254	-
Mycoplasma genitalium	203	560
Ureaplasma parvum	383	560
Ureaplasma urealyticum (T-960)	206	560
Trichomonas vaginalis	218	560
Neisseria gonorrhoeae	329	560
Cytomegalovirus (CMV)	280	560
Candida albicans	310	560
Type 6 Human herpes virus (HHV-6)	277	560
Type 8 Human herpes virus (HHV-8)	293	560
Human Papillomavirus types 51 and 26 (HPV 51, 26)	520	-
Human Papillomavirus types 6 and 11 (HPV 6, 11)	372	-
Bordetella pertussis	419	-
Helicobacter pylori	348	560
Streptococcus pyogenes	435	-
Toxoplasma gondii	187	560
Vibrio cholerae toxicogenous strains	508	-
Epshtein-Barr Virus (EBV)	185	560
Varicella zoster virus (VZV)	269	560
Thermal cycling program 2		
Mycoplasma hominis	310	900
Ureaplasma urealyticum (T-960)+ Ureaplasma parvum	532	900
Herpes simplex virus 1,2 (HSV-1, 2)	261	900
Human Papillomavirus Type 16 (HPV 16)	367	900
Human Papillomavirus Type 18 (HPV 18)	417	900
Thermal cycling program 3		
Gardnerella vaginalis	445	900
Thermal cycling program 4		
Human Papillomavirus types 16, 31, 33, 35, 35H, 52, 58, 67 (HPV 16, 31, 33, 35, 35H, 52, 58, 67)	570/642[2]	-
Human Papillomavirus types 18, 45, 39, 59 (HPV 18, 45, 39, 59)	285-297[3]	-

[2] – for DNA PCR reagent kit HPV 16, 31, 33, 35, 35H, 52, 58, 67 upon presence of HPV 33, 58, 67 DNA in samples the PCR product is 570 bp long; upon presence of HPV 16, 31, 35, 35H, 52 DNA the product is 642 bp long. Both products are amplified in a positive control DNA sample.

[3] – for HPV 18, 45, 39, 59 PCR system the products have similar lengths (HPV 18 – 285, HPV 45 – 291, HPV 39 – 294, HPV 59 – 297 bp, the difference between them can not be seen when using agarose gel electrophoresis.