117587, Moscow, Varshavskoye sh., 125, building 6g
Phone/Fax +7 (495) 980-45-55
Customer service department: +7 (800) 200-75-15
(toll-free within Russia)
E-mail: hotline@dna-technology.ru,
www.dna-technology.ru

FLASH PCR detection Kit
(for fluorescence end-point detection)

Content (for 100 tests)

Reagent	Quantity	
• Paraffin sealed PCR-mix	20 µL	100 tubes
• Taq–polymerase solution	500 µL	2 tubes
• Background buffer	200 µL	1 tube
• Mineral oil	1,0 µL	2 tubes
• Positive control ("C+")	150 µL	1 tube

FLASH PCR kits (for fluorescence end-point detection) are manufactured for 100 or 50 tests (in 0.5 mL and 0.2 mL tubes) depending on their names.

Procedure overview

1. Preparing PCR

1.1. Mark tubes with paraffin sealed PCR-mix (considering negative control ("C–"), positive control ("C+") and two calibration tubes ("BACKGROUND").

1.2. Vortex the tubes with Taq-polymerase solution for 3-5 seconds and spin for 1-3 seconds to collect drops.

1.3. Add 10 µL of Taq-polymerase solution into each tube (except "BACKGROUND" tubes). Avoid paraffin layer break. Add 10 µL of PCR buffer to the "BACKGROUND" tubes.

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+" and "BACKGROUND" tubes.

1.6. Add 5.0 µL of negative control which passed all steps of DNA extraction into "C-" and "BACKGROUND" tubes. Add 5.0 µL of positive control into "C+" marked tubes. Avoid paraffin layer break.

1.7. Spin tubes briefly (1-3 sec) at 1000 rpm.

1.8. Set all tubes in PCR cycler and carry out PCR under conditions showed in tables 1-3 considering 35 µL reaction mix volume. Using Thercyc cycler you need to choose «Precision active regulation» regulation algorithm. Amplification programs correspondence with reagent kits is shown in Table 4.

Note. When using the FLASH PCR detection Kit the multiple usage of backgrounds of the same batch is allowed. The "BACKGROUND" tubes should be stored at at 2-8 °C and out of light. Take the tubes out of refrigerator 1 hour before the run to let the content warm up to room temperature.

2. The PCR and post-PCR analysis

Detection and interpretation of DNA PCR cycling results is performed with the help of "Gene" and "Gene–4" PCR detectors (according to the appliance manual the threshold values are 1.75 – 2.10 for a specific product and 2.50 for internal control), or with the help of gel electrophoresis (see Table 4 and gel electrophoresis manual).

DNA probes used for detection of PCR products of a specific sequence and internal control sample are labeled with fluorescent FAM and HEX labels respectively.

Storage and handling requirements

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

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

PCR Kit shelf life period – 12 months since the date of production.

Contact our customer service department regarding issues of quality of the FLASH PCR detection Kit:
Phone:+7 (800) 200-75-15,
Phone/Fax: +7(495)980-45-55
E-mail: hotline@dna-technology.ru, www.dna-technology.ru
Address:115587, Moscow, Varshavskoye sh., 125g building 6, DNA Technology

Table 1

PCR routine 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	00	94,0	1	00	1
2	94,0 64,0 67,0	0 0 0	5 5 5	94,0 64,0 67,0	0 0 0	50 50 50	5
3	94,0 64,0 67,0	0 0 0	1 5 5	94,0 64,0 67,0	0 0 0	50 50 50	40
4	10,0	…	…	10,0	…	…	Storage

Table 2

PCR routine 2

Block№	Thermocyclers with active temperature adjustment			Thermocyclers without active temperature adjustment			Cycles number
	Temperature, °C	Time		Температура, °C	Temperature, °C		
		min	sec		min	sec	
1	94,0	1	00	94,0	1	00	1
2	94,0 67,0	0 0	5 15	94,0 67,0	0 0	50 50	5
3	94,0 67,0	0 0	1 15	94,0 67,0	0 0	50 50	40
4	10,0	…	…	10,0	…	…	Storage

Table 3

PCR routine 3

Block№	Thermocyclers with active temperature adjustment			Thermocyclers without active temperature adjustment			Cycles number
	Temperature, °C	Time		Температура, °C	Temperature, °C		
		min	sec		min	sec	
1	94,0	1	00	94,0	1	00	1
2	94,0 67,0	0 0	5 15	94,0 67,0	0 0	50 50	5
3	94,0 67,0	0 0	1 15	94,0 67,0	0 0	50 50	30
4	10,0	…	…	10,0	…	…	Storage

DNA PCR product lengths

PCR product	PCR product length, bp	Internal control length, bp
PCR routine 1		
Chlamydia trachomatis	415	900
Mycoplasma genitalium	203	560
Ureaplasma parvum	383	560
Ureaplasma urealyticum (T–960)	206	560
Trichomonas vaginalis	218	560
Type 6 Human herpes virus (HHV–6)	277	560
Type 8 Human herpes virus (HHV–8)	293	560
Cytomegalovirus (CMV)	280	560
Neisseria gonorrhoeae	329	560
Candida albicans	310	560
Bordetella pertussis	419*	560
Legionella pneumophila	383	560
Helicobacter pylori	348	560
Corynebacterium diphtheriae (toxicogenous strains)	251	560
Listeria monocytogenes	67**	560
A group Streptococcus *Streptococcus pyogenes*	455*	560
Toxoplasma gondii	187	560
Epshtein–Barr Virus (EBV)	185	560
Varicella zoster virus (VZV)	269	560
PCR routine 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
Mycobacterium tuberculosis + *Mycobacterium bovis*	330	900
PCR routine 3		
Gardnerella vaginalis	445	900

* – specific DNA PCR products *(Bordetella pertussis, Streptococcus pyogenes)* and internal control have similar lengths with the difference unseen with electrophoretic detection

** – specific DNA PCR products *(Listeria monocytogenes)* are not discriminated in standard 1,5% agarose gels.

115587, Moscow, Varshavskoye sh. 125g building 6,
DNA Technology
Phone/Fax: +7 (495) 980-45-55
Customer service department: +7 (800) 200-75-15
e-mail: hotline@dna-technology.ru
www.dna-technology.ru