



The ENZYME Company

DFS-Taq DNA polymerase

Cat: 101005 500 units

Description DFS (DNA Free Sensitive) Taq DNA Polymerase is a thermostable enzyme of approximately 94kDa isolated from eubacterium *Thermus aquaticus* strain YT-1(1). This unmodified enzyme replicates DNA at 72 °C. The enzyme catalyzes the polymerization of nucleotides into duplex DNA in the 5'-->3' direction in the presence of magnesium ions and possesses a 5'-->3' exonuclease activity. The enzyme is highly purified and is free of nonspecific endo- or exonucleases. Taq DNA polymerase leaves single 3'-dA nucleotide overhangs on their reaction products.

Performance and purity tests: Taq DNA polymerase effectively directs PCR with the template up to 5 kb in length. Enzyme was tested on the absence of endonuclease and nickase activities. No traces of bacterial DNA were detected in PCR reaction with "no template" test with the primers complementary to the conservative region of 16S ribosomal gene.

The following tests are performed with each lot of DFS Taq DNA polymerase:

- PCR with various templates – human and bovine genomic DNA, Phage Lambda DNA;
- Exo-endo nucleases contamination tests;
- "no primers" test with Lambda DNA cycling without primers;
- "no template" test with the primers complementary to the conservative region of 16S bacterial ribosomal genes;
- storage (3 days at room temperature) test – no change in performance.

Applications DNA-free Taq DNA polymerase is suitable for all regular applications – PCR, primer extension reactions etc. DFS Taq DNA polymerase is free from bacterial DNA and it is especially recommended for the work with bacterial DNA.

Sensitivity of PCR reaction with DFS Taq DNA polymerase in the optimal conditions is very high – in some reactions less than 6 DNA molecules were detected. Enzyme has a very good performance in single-copy gene PCR from genomic mammalian DNA. In contrast to Bioron enzyme, Taq DNA polymerases from the variety of suppliers contain contaminating DNA, with DNA-contaminated Taq DNA polymerase one should be ready to observe false-positive PCR results in some cases.

Concentration 5000 units/ml

Storage buffer 10 mM K-phosphate, pH 7.4, 0.1 mM EDTA, 50% glycerol, 0.1% Triton X-100, 0.1% Tween 20.

Unit definition One unit of activity is the amount of enzyme required to incorporate 10 nmoles of dNTP into acid-insoluble DNA fraction in 30 minutes at 72°C.

Reaction buffer (x10) "incomplete": 160 mM (NH₄)₂SO₄, 670mM TrisHCl pH8,8, 0,1% Tween-20

Reaction buffer (x10) "complete": 160 mM (NH₄)₂SO₄, 670mM TrisHCl pH8,8, 0,1% Tween-20, 25mM MgCl₂

Reaction buffer (x10) "complete II KCl": 500 mM KCl, 100mM TrisHCl pH8,8, 0,1% Tween-20, 15mM MgCl₂

Additionally provided: 1 Tube MgCl₂ (100 mM)

Recommended MgCl₂ concentration 1,5mM – 6mM

Storage conditions Recommended storage temperature is -20°C, meanwhile, the enzyme is stable at room temperature at least for 3 days without any loss of activity

Reference: 1.Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I. (1980) *Biokhimiya* **45**, 644(Rus)

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Catalog #	conc.	Pack size
101005	5 u/μl	500 u
101025	5 u/μl	2500 u
101100	5 u/μl	10000 u
101500	5 u/μl	50000 u

Pipetting scheme and Thermocycler protocol:

Components	Volume / 50μl PCR-Reaction	Final concentration
10 x PCR-Buffer	5 μl	1 x
dNTP-Mix (40mM)	1 μl	800 μM (200μM each)
Upstream Primer	variabel	0,1-0,5 μM
Downstream Primer	variabel	0,1-0,5 μM
DFS-Taq DNA Polymerase	0,25-1,0 μl	1,25-5,0 units
Template DNA	variabel	10 to 500ng /reaction
Sterile dest. water	Adjust to 50 μl final volume	

Separate MgCl₂ solution can be used, if incomplete buffer is used, or if you have to **titrate MgCl₂** for optimal PCR results:

Final MgCl₂ conc. mM	1,5	2	2,5	3	3,5	4	4,5	5	5,5	6
Volume in μl of 100 mM MgCl₂ per 50 μl reaction	0,75	1	1,25	1,5	1,75	2	2,25	2,5	2,75	3

Thermocycler Protocol

step	time	temperature
initial denaturation	2 minutes	94 °C
30 cycles:		
denaturation	10 seconds	94 °C
annealing	20 seconds	55 °C
extension	1 minute	72 °C

Notes:

Program the cycler according to manufacturers instructions. Each program should start with an initial denaturation step at 94 °C for 2 to max. 5 min.

Recommended elongation time is 1 min per 1kb of target.

For maximum yield and specificity, temperatures (annealing) and cycling times should be optimized for each new template target or primer pair.

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