DFS HotTaq DNA Polymerase

Description: DFS HotTaq DNA Polymerase is a thermostable enzyme of approximately 94 kDa, isolated from eubacterium *Thermus aquaticus* strain YT-1 bound to anti-*Taq* DNA polymerase monoclonal antibodies. Polymerase activity is blocked during set-up of the PCR at ambient temperature (20 – 22 °C). The inhibition is completely reversed when the temperature is increased above 70 °C The enzyme catalyzes the polymerization of nucleotides into duplex DNA in the 5' → 3' direction in the presence of magnesium ions and shows 5' → 3' exonuclease activity. The enzyme is highly purified and free of nonspecific endo- or exonucleases.

Content

Ref No.	S101004	101004	101024	color
DFS-HotTaq DNA Polymerase	**Sample size**	**500 units**	**2500 units**	**Blue**
Incomplete NH_4 * Reaction Buffer (10x)	**1,8 mL**	**1.8 mL**	**5x 1.8 mL**	**Red**
Complete NH_4 ** Reaction Buffer (10x)	**1,8 mL**	**1.8 mL**	**5x 1.8 mL**	**yellow**
Complete KCl * Reaction Buffer (10x)**	**1,8 mL**	**1.8 mL**	**5x 1.8 mL**	**black**
$MgCl_2$ 100 mM	**1 mL**	**1 mL**	**5x 1 mL**	**green**
Datasheet	**1**	**1**	**1**	**--**

* Incomplete NH_4 Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, free of $MgCl_2$.

** Complete NH_4 Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, 25 mM $MgCl_2$.

*** Complete KCl Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, 15 mM $MgCl_2$.

Applications: DFS HotTaq DNA Polymerase is suitable for all regular applications - PCR, primer extension reactions etc. DFS HotTaq is free of bacterial DNA and therefore suitable for detection of pathogen bacteria. DFS HotTaq DNA Polymerase effectively directs PCR with templates up to 5 kb in length.

Concentration: 5 units/µL

Sensitivity: detection of ≥ 10 DNA molecules

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

Additionally provided: 1 tube $MgCl_2$ (100 mM)

Recommended $MgCl_2$ concentration: 1.5 mM – 6 mM

Quality Control

- 98% protein homogeneity in 10% SDS-PAGE
- No detectable exo-/endonuclease activities
- PCR amplification tests with different templates
- PCR amplification tests without templates as Negative Control
- “no template” test with primers complementary to a region auf 16S bacterial ribosomal genes
- Hotstart efficiency test showing effective blockage by AntiTaq

Storage condition: -20 °C

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Pipetting scheme and thermocycler protocol

Components	Volume / 50 µL Reaction	Final concentration
10 x PCR-Buffer	5 µL	1 x
dNTP-Mix (10 mM each)	1 µL	200 µM each
Upstream Primer	variable	0.1 - 0.5 µM
Downstream Primer	variable	0.1 - 0.5 µM
DFS HotTaq DNA Polymerase	0.25 - 1.0 µL	1.25 - 5.0 units
Template DNA	variable	10 to 500 ng/reaction
Sterile dest. water	Adjust to 50 µL final volume	

Separate $MgCl_2$ solution can be used for optimization. If incomplete buffer is used **titrate $MgCl_2$** for optimal PCR results with following recommendation (see table):

Final $MgCl_2$ conc. [mM]	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
Volume [µL] of 100 mM $MgCl_2$/ 50 µL	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 - 68 °C *
extension	1 minute	72 °C

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers.

Notes:
Program the cycler according to the manufacturer's instructions.
Each program should start with an initial denaturation step at 94 °C for 2 to max. 5 min.
Recommended elongation time: 1 min/ 1 kb 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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