HighTaq DNA Polymerase

Description: HighTaq DNA Polymerase is a novel genetically engineered version of *Taq* DNA polymerase which provides high robustness against inhibitors (e.g. bio-molecules like polysaccharides, fats and proteins). The performance of the enzyme ensures high sensitivity and reliable amplification. The polymerase replicates DNA at 72 °C. It catalyses the polymerization of nucleotides into duplex DNA in 5' → 3' direction and possess a 5' → 3' exonuclease activity. High Taq DNA Polymerase is available as high concentrated solution with 75 U/µl.

Content

Ref No.	S111105 (5 U/µl)	111105 (5 U/µl)	111125 (5 U/µl)	111106 (75 U/µl)	color
HighTaq DNA Polymerase	Sample size	500 units	2500 units	1000 units	blue
Incomplete NH_4 * Reaction Buffer (10x)	1.8 mL	1.8 mL	5x 1.8 mL	2x 1.8 mL	red
Complete NH_4 ** Reaction Buffer (10x)	1.8 mL	1.8 mL	5x 1.8 mL	2x 1.8 mL	yellow
Complete KCl *** Reaction Buffer (10x)	1.8 mL	1.8 mL	5x 1.8 mL	2x 1.8 mL	black
$MgCl_2$ 100 mM	1 mL	1 mL	5x 1 mL	2x 1 mL	green
Datasheet	1	1	1	1	--

* Incomplete NH_4 Reaction Buffer (10x): 160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl (pH 8,8), 0.1 % Tween 20

** Complete NH_4 Reaction Buffer (10x):160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl (pH 8,8), 0.1 % Tween 20, 25 mM $MgCl_2$.

*** Complete KCl Reaction Buffer (10x): 500 mM KCl, 100 mM Tris-HCl (pH 8,8), 0.1 % Tween 20, 15 mM $MgCl_2$.

Applications: HighTaq DNA Polymerase is suitable for all regular applications but especially for those where inhibitors might decrease the performance. HighTaq DNA Polymerase effectively directs PCR with the template up to 5 kb in length. High Taq DNA Polymerase high concentrated (75 U/µl) is recommended for lyophilization.

Concentration: 5 units/µL

Sensitivity: detection of ≥ 6 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 m – 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

Storage condition: -20 °C

Pipetting scheme and thermocycler protocol:

Components	Volume / 50 µL PCR-Reaction	Final concentration
10 x PCR-Buffer	5 µL	1x
dNTP-Mix (40 mM)	1 µL	800 µM (200 µM each)
Upstream Primer	variable	0.1 - 0.5 µM
Downstream Primer	variable	0.1 - 0.5 µM
HighTaq 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.