Taq DNA Polymerase

From Thermus aquaticus YT-1, recombinant (E.coli)

Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase, Taq Pol, EC 2.7.7.7
Product code A5186

Description

Taq DNA Polymerase is a thermostable enzyme of approximately 94 kDa 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 nucletide overhangs on their reaction products.

Concentration 5000 units/ml

Storage buffer 10 mM potassium 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 $(NH_4)_2SO_4$, 670 mM Tris · HCl pH 8.8, 0.1 % Tween® 20

Reaction buffer (x10) complete:
160 mM $(NH_4)_2SO_4$, 670 mM Tris · HCl pH 8.8, 0.1 % Tween® 20, 25 mM $MgCl_2$

Reaction buffer (x10) complete II KCl:
500 mM KCl, 100 mM Tris · HCl (pH 8.8), 0.1 % Tween® 20, 15 mM $MgCl_2$

+ 1 Tube $MgCl_2$ (100mM)

Recommended $MgCl_2$ concentration 1.5 mM - 6 mM

Storage conditions The recommended storage temperature is -20°C A test was performed showing that the enzyme is stable at room temperature for at least 3 days without any loss of activity.

Anwendung und Literatur

(1) Kaledin, A.S. *et al.* (1980) *Biokhimiya* **45**, 644 (Rus).

Pipetting Scheme

Reagents	Volume	Final concentration
10x PCR reaction buffer	5 µl	1X
dNTP mix (40 mM)	1 µl	200 µM (of each dNTP)
Upstream Primer	variable	0.1-0.5 µM
Downstream Primer	variable	0.1-0.5 µM
Taq DNA Polymerase	0.25 - 1.0 µl	1.25 - 5.0 U
Template DNA	variable	10 - 500 ng / reaction
Sterile ddH_20	ad 50 µl	
Final volume	**50 µl**	

Separate $MgCl_2$ solution can be used, if incomplete buffer is used, or if you have to titrate $MgCl_2$ for optimal PCR results.

Final $MgCl_2$ conc. [mM]	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
Volume of 100 mM $MgCl_2$ [µl]	0.75	1	1.25	1.5	1.75	2	2.25	2.5	2.75	3

Thermocycler Protocol

Step	Temperature	Time	Cycle No.
Initial denaturation	94°C	2 min	1x
Denaturation	94°C	10 sec	
Annealing	55°C	20 sec	30X
Elongation	72°C	1 min	
Final elongation	72°C	10 min	1x

Notes
Program the cycler according to the manufacturer's instructions. Each program shall start with an initial denaturation step at 94°C for 2 to max. 5 min.
Recommended elongation time is 1 min per 1 kb of template
For maximum yield and specificity, temperature (annealing) and cycling times shall be optimized for each new template or pair of primers.