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Taq DNA polymerase

(Catalogue number T032, T033, T034) rev. 07/2025

Description

Taq DNA polymerase is a thermostable enzyme isolated from *Thermus aquaticus*. The enzyme catalyzes the synthesis of the complementary DNA strand in the 5´->3´ direction and also possesses a 5´->3´ exonuclease activity. During amplification of DNA fragments, Taq polymerase adds an adenosine overhang to the 3´ end. This can be utilized for cloning of PCR-generated DNA fragments. The advantage of the enzyme is its high processivity [amplification of 1000 base pairs (bps) takes < 1 min]. The disadvantage of the enzyme is that it lacks a 3´->5´ exonuclease proofreading activity, and this accounts for a high error rate (about 1 error per 105 - 106 base pairs). The major usage of the enzyme is in diagnostic analysis for amplification of DNA fragments up to 5000 bps.

Technical data

Components and packaging

- Taq DNA polymerase is supplied at a concentration of 5 U/µl. Basic packaging is 1 tube with 500 U/100 µl (**T032**), 5 tubes with 500 U/100 µl (**T033**), or 10 tubes with 500 U/100 µl (**T034**).
- Each tube of Taq DNA polymerase is accompanied by a tube with 10x PCR Blue buffer, which exhibits higher tolerance to suboptimal Mg2+ concentrations (blue cap, cat. no. **T058**).

To optimize $MgCl_2$ concentration, order 10x conc. PCR Blue Buffer without $MgCl_2$ and 25 mM $MgCl_2$ in a separate tube (Cat. no. **T059**).

Storage

- At temperature -20°C ± 5°C. Material can be repeatedly defrosted.

Composition

- Storage buffer for Taq DNA polymerase: 20 mM HEPES (pH 7.9 at 25°C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, stabilizers, 50% glycerol.
- 10x concentrated PCR Blue Buffer: 750 mM Tris-HCl, pH 8.8 (at 25 °C), 200 mM $(NH_4)_2SO_4$, 1% Tween 20, 25 mM $MgCl_2$.

Activity

- One unit of Taq DNA polymerase is defined as the amount of enzyme, which catalyzes incorporation of 10 nmol dNTPs within 30 min at 72°C into trichloracetic acid precipitable material. Reaction conditions are as follows: 10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 200 µM dATP, dCTP, dGTP and [α-^{32}P]dTTP, 50 µg/ml activated salmon testes DNA, 0.5 µM primer and 0.2 – 0.5 U of enzyme in the volume of 50 µl.

Purity and quality control

- The purity of Taq DNA polymerase is tested using SDS-PAGE. After staining with Coomassie blue, the enzyme migrates as the major band of 94 kDa. The material is nuclease-free.
- Each batch of Taq DNA polymerase is tested for its ability to amplify DNA fragment from mammalian genomic DNA by PCR. The results are verified by electrophoresis in agarose gel in the presence of ethidium bromide; only DNA band of the expected size is present.

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Protocol

Basic protocol

The protocol described below can be used for routine PCR. However, in some cases, reaction conditions must be optimized, mainly the annealing temperature and the concentration of $MgCl_2$.

1. In thin-wall test tubes the following components are mixed[1]:

	PCR in 50 µl	**Final concentration**
10x PCR Blue Buffer with 25 mM $MgCl_2$[2]	5 µl	1x react. buffer with 2.5 mM $MgCl_2$
PCR dNTP mix (10 mM each) (Cat. No. P041)	1 µl	0.2 mM dNTP each
5´ primer (50 µM)	0.5 µl	0.5 µM
3´ primer (50 µM)	0.5 µl	0.5 µM
Taq DNA polymerase (5U/µl)	0.5 ul	2.5 U (0.05 U/µl)
Template DNA (1 ng/µl - 1 µg/µl)	1 ul	0.02 ng/µl – 0.02 µg/µl
PCR H_2O (Cat. No. P042)	41.5 ul	

[1] When more DNA samples are tested with the same primers, it is convenient to prepare so called Master Mix in which the volumes of individual components are multiplied by the number of DNA samples tested. Then, 49 µl aliquots of the Master Mix are distributed into the PCR tubes, followed by addition of tested DNA (1 µl) into each tube.

[2] If unsatisfactory results are obtained, PCRs with different concentrations of $MgCl_2$ should be performed (see below).

2. Samples are homogenized and spun down.

3. If a cycler without heating lid is used, PCR oil 25 µl (Cat. No. P043) is added.

4. The thermal cycler is programmed according to the manufacturer´s instructions. A typical cycling program is as follows:

	Temperature	**Time**	**Number of cycles**
Initial denaturation	94°C	3 min	1
Denaturation	94°C	30 s	25-35
Annealing of primers	55-68°C[1]	30 s	
Extension	72°C	1 min per 1 kb	
Final extension	72°C	10 min	1
Cooling	4°C		

[1] Should be determined experimentally; usually 5°C below melting temperature of the primers.

5. After PCR, samples are mixed with loading buffer (Cat. No. P048, P064 or P066) and analyzed by electrophoresis in agarose gel in the presence of ethidium bromide. Alternatively, the samples can be stored at -20°C ± 5°C.

Optimization of $MgCl_2$ concentration

$MgCl_2$ at a 2.5 mM final concentration is suitable for most PCRs with Taq DNA polymerase. However, if amplification of nonspecific DNA fragments is observed, the optimal Mg2+ concentration for the given PCR should be determined. To this end, 10x reaction buffer without $MgCl_2$ and 25 mM $MgCl_2$ can be ordered (Cat. No. T059).

1. Preparation of Master Mix **without $MgCl_2$ by mixing the following components:**

10x PCR Blue Buffer without $MgCl_2$	40 µl
PCR dNTP mix (10 mM each)	8 µl
5´ primer (50 µM)	4 µl
3´ primer (50 µM)	4 µl
Taq DNA polymerase (5U/µl)	4 µl
Template DNA (1 ng/µl - 1 µg/µl)	8 µl
PCR H_2O	252 µl
Total volume	**320 µl**

2. Master mix is thoroughly mixed, centrifuged briefly, and 40 µl aliquots are distributed into 7 PCR tubes.

3. $MgCl_2$ and PCR H_2O are added into the PCR Master mixes as follows:

Tube No.	**25 mM $MgCl_2$**	**PCR H_2O**	**Final $MgCl_2$ concentration**
1	2 µl	8 µl	1.0 mM
2	3 µl	7 µl	1. mM
3	4 µl	6 µl	2.0 mM
4	5 µl	5 µl	2.5 mM
5	6 µl	4 µl	3.0 mM
6	8 µl	2 µl	4.0 mM
7	10 µl	0 µl	5.0 mM

4. PCR is performed as above and the samples are analyzed by electrophoresis in agarose gel in the presence of ethidium bromide. Optimal concentration of $MgCl_2$ for a given PCR is thus determined.

Cat. No.	Product name and specification	Amount
T032	Taq DNA polymerase	500U
T033	Taq DNA polymerase	5x 500U
T034	Taq DNA polymerase	10x 500U
T058	10x PCR Blue Buffer	1.5 ml
T059	10x PCR Blue Buffer without $MgCl_2$+$MgCl_2$	1.5 ml + 0.5 ml