

Taq DNA polymerase Unis

(Catalogue number T037, T038, T039)

rev. 07/2025

Description

Taq DNA polymerase Unis is an alternative to Taq DNA polymerase (Cat. No. T032-T034). Difference between these two products is in difference in enzyme storage buffer which enhances stability and efficiency of the enzyme. Occasional amplification of nonspecific fragments can be prevented by enhanced dilution of the enzyme.

Taq DNA polymerase

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 Unis is supplied at a concentration of 5 U/μl. Basic packaging is 1 tube with 500 U/100 μl (**T037**), 5 tubes with 500 U/100 μl (**T038**) or 10 tubes with 500 U/100 μl (**T039**).
- Each tube of Taq DNA polymerase Unis 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 MgCl₂ concentration, order 10x conc. PCR Blue Buffer without MgCl₂ and 25 mM MgCl₂ 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 Unis: 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 (NH₄)₂SO₄, 1% Tween 20, 25 mM MgCl₂.

Activity

- One unit is defined as the amount of enzyme, that catalyzes incorporation of 10 nmol dNTPs within 30 min at 72°C into trichloroacetic acid precipitable material. Reaction conditions are as follow: 10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl₂, 200 μM dATP, dCTP, dGTP and [α-³²P]dTTP, 50 μg/ml denatured cDNA, 0.5 μM primer and 0.2 – 0.5 U of enzyme in a volume of 50 μl.

Purity and quality control

- Purity of Taq DNA polymerase Unis 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 Unis 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 MgCl₂.

1. In thin-wall test tubes the following components are mixed¹:

	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 Unis (5U/µl)	0.5 µl	2.5 U (0.05 U/µl)
Template DNA (1 ng/µl - 1 µg/µl)	1 µl	0.02 ng/µl – 0.02 µg/µl
PCR H ₂ O (Cat. No. P042)	41.5 µl	

¹ When more DNA samples are tested with the same primers, it is convenient to prepare the 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 MgCl₂ should be performed (see below).

2. Samples are homogenized and spun down.

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

4. Thermal cycler is programmed according to the manufacturer's instructions. A typical cycling program is:

	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.

Optimization of MgCl₂ concentration

MgCl₂ at a 1.5 mM final concentration is suitable for most PCRs with Taq DNA polymerase Unis. However, if amplification of nonspecific DNA fragments is observed, optimal Mg²⁺ concentration for given PCR should be determined. To this end 10x PCR Blue Buffer without MgCl₂ and 25 mM MgCl₂ can be ordered (Cat. No. T059).

1. Preparation of Master Mix **without MgCl₂** 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 Unis (5U/µl)	4 µl
Template DNA (1 ng/µl - 1 µg/µl)	8 µl
PCR H ₂ O	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. MgCl₂ and PCR H₂O is added into PCR Master mixes as follows:

Tube No.	25 mM MgCl ₂	PCR H ₂ O	Final MgCl ₂ concentration
1	2 µl	8 µl	1.0 mM
2	3 µl	7 µl	1.5 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 MgCl₂ for given PCR is thus determined.

Cat. No.	Product name and specification	Amount
T037	Taq DNA polymerase Unis	500U
T038	Taq DNA polymerase Unis	5x 500U
T039	Taq DNA polymerase Unis	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

