

## Taq DNA polymerase 1.1

(Catalogue number T112, T113, T114)

rev. 07/2025

### Description

This product is a modification of Taq DNA polymerase Unis (Cat. No. T037-T 039). Difference between these two products rely in lower concentration of the polymerase (1 U/μl), which makes easier pipetting of the enzyme. Furthermore, the enzyme is supplied with 10x concentrated PCR Blue buffer. This buffer contains ammonium sulfate, which contributes to broader tolerance of MgCl<sub>2</sub> and lower production of nonspecific enzymes.

#### **Taq DNA polymerase**

*Taq DNA polymerase is a thermostable enzyme isolated from Thermus aquaticus. The enzyme catalyzes synthesis of complementary DNA strand in the 5'→3' direction and also possesses a 5'→3' exonuclease activity. During amplification of DNA fragments, Taq polymerase adds at 3' end an adenosine overhang. This can be utilized for cloning of PCR-generated DNA fragments. Advantage of the enzyme is its high processivity (amplification of 1000 base pairs takes < 1 min). Disadvantage of the enzyme is that it lacks a 3'→5' exonuclease proofreading activity and this accounts for high error rate [about 1 error to 10<sup>5</sup> - 10<sup>6</sup> base pairs (bps)]. 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 1.1 is supplied at a concentration 1 U/μl. Basic packaging is 1 tube with 500 U/500 μl (**T112**), 5 tubes with 500 U/500 μl (**T113**) or 10 tubes with 500 U/500 μl (**T114**).
- Each tube of Taq DNA polymerase 1.1 is accompanied by a tube with 10x concentrated PCR Blue buffer containing MgCl<sub>2</sub> (1.5 ml).

If different concentration of MgCl<sub>2</sub> is required, a tube with 10x concentrated PCR Blue Buffer without MgCl<sub>2</sub> (1.5 ml) and a tube with 25 mM MgCl<sub>2</sub> (0.5 ml) should be ordered (Cat. No. **T059**).

#### **Storage**

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

#### **Composition**

- Storage buffer: 20 mM Tris-HCl (pH 8.0 at 25°C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Nonidet P-40, 0.5% Tween 20, 50% glycerol.
- 10x PCR Blue Buffer: 750 mM Tris-HCl, pH 8.8 (at 25°C), 200 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1% Tween 20, 25 mM MgCl<sub>2</sub>.

#### **Activity**

- One unit is defined as the amount of enzyme, which 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 MgCl<sub>2</sub>, 200 μM dATP, dCTP, dGTP and [α-<sup>32</sup>P]dTTP, 50 μg/ml denatured cDNA, 0.5 μM primer and 0.5 U of enzyme in the volume of 50 μl.

#### **Purity and quality control**

- Purity of Taq DNA polymerase 1.1 is tested by SDS-PAGE. Enzyme migrates as a major band of 94 kDa. Material is nuclease free.
- Each batch of Taq DNA polymerase 1.1 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.

# Taq DNA polymerase 1.1

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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 annealing temperature and concentration of MgCl<sub>2</sub>.

1. In a thin-wall PCR test tube the following components are mixed<sup>1</sup>:

	PCR in 50 µl	Final concentration
10x PCR Blue Buffer with 25 mM MgCl <sub>2</sub> <sup>2</sup>	5 µl	1x react. buffer with 2.5 mM MgCl <sub>2</sub>
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 1.1. (1U/µl)	2.5 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 <sub>2</sub> O (Cat. No. P042)	39.5 µl	

<sup>1</sup> 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.

<sup>2</sup> If unsatisfactory results are obtained, PCRs with different concentrations of MgCl<sub>2</sub> 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 <sup>1</sup>	30 s	
Extension	72°C	1 min per 1 kb	
Final extension	72°C	10 min	1
Cooling	4°C		

<sup>1</sup> 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, P066 or P065) and analyzed by electrophoresis in agarose gel in the presence of ethidium bromide. Alternatively, the samples can be stored at -20°C.

### Optimization of MgCl<sub>2</sub> concentration

MgCl<sub>2</sub> at a 2.5 mM final concentration is suitable for most PCRs with Taq DNA polymerase 1.1. However, if amplification of nonspecific DNA fragments is observed, optimal Mg<sup>2+</sup> concentration for given PCR should be determined. To this end 10x PCR Blue Buffer without MgCl<sub>2</sub> and 25 mM MgCl<sub>2</sub> can be ordered (Cat. No. T59).

1. Preparation of Master Mix **without MgCl<sub>2</sub>** by mixing the following components:

10x PCR Blue Buffer without MgCl <sub>2</sub>	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 1.1 (1U/µl)	20 µl
Template DNA (1 ng/µl - 1 µg/µl)	8 µl
PCR H <sub>2</sub> O	236 µl
<b>Total volume</b>	<b>320 µl</b>

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

3. MgCl<sub>2</sub> and PCR H<sub>2</sub>O is added into PCR Master mixes as follows:

Tube No.	25 mM MgCl <sub>2</sub>	PCR H <sub>2</sub> O	Final MgCl <sub>2</sub> 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 MgCl<sub>2</sub> for given PCR is thus determined.

Cat. No.	Product name and specification	Amount
T112	Taq DNA polymerase 1.1	500U
T113	Taq DNA polymerase 1.1	5x 500U
T114	Taq DNA polymerase 1.1	10x 500U
T059	10x conc. PCR Blue Buffer without MgCl <sub>2</sub> +MgCl <sub>2</sub>	1.5 ml + 0.5 ml

