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Combi Taq DNA polymerase

(Catalogue number C205, C206, C207) rev. 01/2022

Description

Combi Taq DNA polymerase is a modification of Taq DNA polymerase Unis (Cat. No. T037-T039). The enzyme is supplemented with monoclonal antibody anti-Taq, which allows **"hot-start"** PCR. Furthermore, the polymerase is present at lower concentration (1U/µl), which simplifies pipetting of the enzyme.

Hot start

- This products contains monoclonal antibody anti-Taq, which binds specifically to Taq DNA polymerase and blocks its enzymatic activity until the first denaturation phase of PCR. At 94°C the antibody is irreversibly denatured and the activity of the polymerase is restored. This decreases formation of nonspecific DNA fragments during PCR.

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 10^5 - 10^6 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

- Combi Taq DNA polymerase is supplied at a concentration 1 U/µl. Basic packaging contains 1 tube with 500 U/500 µl (C205), 5 tubes with 500 U/500 µl (C206) or 10 tubes with 500 U/500 µl (C207).
- Each tube of Combi Taq DNA polymerase is accompanied by a tube with 10x concentrated react buffer with $MgCl_2$ (1.5 ml). If different concentration of $MgCl_2$ is required, a tube with 10x concentrated reaction buffer without $MgCl_2$ (1.5 ml) and a tube with 25 mM $MgCl_2$ (0.5 ml) should be ordered (Cat. No. T035).

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, 0.02 mM EDTA, 0.2 mM DTT, 0.1% Nonidet P-40, 0.1% Tween 20, 50% glycerol.
- 10x reaction buffer: 100 mM Tris-HCl, pH 8.8 (at 25°C), 500 mM KCl, 1% Triton X-100, 15 mM $MgCl_2$.

Activity

- One unit 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 follow: 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 denatured cDNA, 0.5 µM primer and 0.2 – 0.5 U of enzyme in the volume of 50 µl.

Purity and quality control

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

Cat. No.	Product name and specification	Amount
C205	Combi Taq DNA polymerase	500U
C206	Combi Taq DNA polymerase	5x 500U
C207	Combi Taq DNA polymerase	10x 500U
T035	10x conc. Taq buffer without $MgCl_2$+$MgCl_2$	1.5 ml + 0.5 ml

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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 $MgCl_2$.

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

	PCR in 50 µl	**Final concentration**
10x reaction buffer with $MgCl_2$[2]	5 µl	1x react. buffer with 1.5 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
Combi Taq DNA polymerase (1U/µl)	2.5 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)	39.5 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 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, P062, 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 $MgCl_2$ concentration

$MgCl_2$ at a 1.5 mM final concentration is suitable for most PCRs. However, if amplification of nonspecific DNA fragments is observed, optimal Mg^{2+} concentration for given PCR should be determined. To this end 10x reaction buffer without $MgCl_2$ and 25 mM $MgCl_2$ can be ordered (Cat. No. T035).

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

10x reaction 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
Combi Taq DNA polymerase (1U/µl)	20 µl
Template DNA (1 ng/µl - 1 µg/µl)	8 µl
PCR H_2O	252 µl

Total volume	**336 µl**

2. Master mix is thoroughly mixed, centrifuged briefly and 42 µl aliquots are distributed into 7 PCR tubes.
3. $MgCl_2$ and PCR H_2O is added into PCR Master mixes as follows:

Tube No.	**25 mM $MgCl_2$**	**PCR H_2O**	**Final $MgCl_2$ concentration**
1	1 µl	7 µl	0.5 mM
2	2 µl	6 µl	1.0 mM
3	3 µl	5 µl	1.5 mM
4	4 µl	4 µl	2.0 mM
5	5 µl	3 µl	2.5 mM
6	6 µl	2 µl	3.0 mM
7	8 µl	0 µl	4.0 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 given PCR is thus determined.