Top-Bio s.r.o.

Tel: +420 603 476 934
E-mail: top-bio@top-bio.cz
www.top-bio.com

Aptamer-Taq DNA polymerase, 5 U/µl

(Catalogue number A025, A026, A027) rev. 01/2022

Description

Aptamer-Taq DNA polymerase, 5 U/µl is a modification of Combi Taq DNA polymerase (Cat. No. C205-C207) in which anti-Taq DNA aptamer is used instead of anti-Taq monoclonal antibody. When compared to antibody the aptamer has several advantages: (**1**) it is not irreversibly denatured at temperature upto 100°C, (**2**) has low molecular weight, is prepared synthetically, and has well defined composition, (**3**) is not contamined with components of mammalian cells, (**4**) is resistant to action of proteases and allows preparation of PCR mixes at room temperature, and (**5**) is less expensive.

Hot start

- This products contains DNA aptamer anti-Taq, which binds specifically to Taq DNA polymerase and blocks its enzymatic activity. At temperature over 50°C aptamer is released and enzymatic activity of Taq DNA 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 DNA 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

- Aptamer-Taq DNA polymerase, 5 U/µl is supplied at a concentration 1 U/µl. Basic packaging contains 1 tube with 500 U/100 µl (A028), 5 tubes with 500 U/100 µl (A029) or 10 tubes with 500 U/100 µl (A030).
- Each tube of Aptamer-Taq DNA polymerase, 5 U/µl 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 PCR Blue Buffer without $MgCl_2$ (1.5 ml) and a tube with 25 mM $MgCl_2$ (0.5 ml) should be ordered (Cat. No. T059).

Storage

- At temperature -20°C ± 5°C. Material can be repeatedly defrosted. For short time (days) it is possible to store at temperature up to 35°C.

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 reaction buffer: 750 mM Tris-HCl, pH 8.8 (at 25°C), 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20, 25 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.

Quality control

- Purity of Aptamer-Taq DNA polymerase, 5 U/µl is tested by SDS-PAGE. Enzyme migrates as a major band of 94 kDa. Material is nuclease free.
- Each batch of Aptamer-Taq DNA polymerase, 5 U/µl is tested for its ability to amplify DNA fragment from mammalian genomic DNA, isolated with DEP-25 DNA extraction kit (Cat. No. D225-D227), 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.

Aptamer-Taq DNA polymerase, 5 U/µl

(Catalogue number A025, A026, A027)

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 25 mM $MgCl_2$[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
Aptame-Taq DNA polymerase, 5 U/µl	0.5 ul	2.5
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 the 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 P064) 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 2.5 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 conc. PCR Blue buffer without $MgCl_2$ and 25 mM $MgCl_2$ can be used (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
Aptamer-Taq DNA polymerase (1U/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 thoroughlyand shortlz mixed, centrifuged briefly and 40 µl aliquots are distributed into 7 PCR tubes.

3. $MgCl_2$ and PCR H_2O is added into 40 µl aliquotes 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	1.5 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 $MgCl_2$ for given PCR is thus determined.

Cat. No.	Product name and specification	Amount
A025	Aptamer-Taq DNA polymerase	500 U
A026	Aptamer-Taq DNA polymerase	5x 500 U
A027	Aptamer-Taq DNA polymerase	10x 500 U
T059	10x conc. PCR Blue buffer without $MgCl_2$+$MgCl_2$	1.5 ml + 0.5 ml