Catalog no. PC-Taq01
Size: 1000 U

Concentration: 5 U/µl
Storage: Store at -20°C.
Shipping: Shipped on dry/blue ice.
Shelf Life: 12 Months

Unit Definition

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTP into acid-insoluble form in 30 minutes at 72ºC.

Purity

≥98% (assessed by SDS-PAGE with Coomassie blue staining)

Source

An E. coli strain expressing a Taq DNA Polymerase gene from *Thermus aquaticus* YT-1.

Product description:

Taq DNA Polymerase is a highly thermostable DNA polymerase (thermophilic bacterium Thermus aquaticus), which possesses a 5´→3´ polymerase activity and a 5´flap endonuclease activity. The enzyme catalyzes 5'→3' synthesis of DNA in the presence of magnesium, with no detectable 3'→5' exonuclease (proofreading) activity, and possesses 5'→3' exonuclease activity. Recombinant Taq DNA Polymerase is ideal for standard PCR applications and can amplify up to 5 kb fragment length or shorter.

Applications

• Routine PCR amplification of DNA fragments up to 5 kb
• Generation of PCR product for TA cloning.
• Colony PCR
• DNA sequencing
• Microarray Analysis

Content

- **5 U/µl Taq polymerase** in 100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.5% Tween® 20, and 30% v/v glycerol.
- **10X PCR Buffer**

  100 mM Tris-HCl (pH 8.3 @25°C), 500 mM KCl, 15 mM $MgCl_2$,

Reaction Conditions:

1X Standard Taq Reaction Buffer, DNA template, primers, 200 µM dNTPs (not included), and 2.5 units of Taq DNA Polymerase in a total reaction volume of 25 µl.

We recommend using Taq DNA Polymerase (ProCyto Labs) at a concentration of 5 units/ µl reaction. However, the optimal concentration of Taq DNA Polymerase may range from 5–50 units/ml (0.25–5 units/50 µl reaction) according to different applications.

PCR

The polymerase chain reaction (PCR) is a robust and sensitive technique used for DNA amplification. A typical amplification reaction includes template DNA, a thermostable DNA polymerase, two oligonucleotide primers, deoxynucleotide triphosphates (dNTPs), reaction buffer, and magnesium. Once assembled, the reaction is placed in a thermal cycler for different cycles of amplification. Taq DNA Polymerase is an enzyme widely used in PCR. The reaction protocols are provided to ensure successful PCR using ProCyto Labs Taq DNA Polymerase. These protocols are followed for routine PCR reactions. Amplification of templates with high GC content, high secondary structure, low template concentrations, or amplicons greater than 5 kb will need further optimization.

Reaction set up using ProCTaq

Place all reaction components on ice and transfer the reaction quickly to a thermocycler preheated to the denaturation temperature (95°C).

Protocol

1. Keep all the solutions on ice for thawing and gently vortex.
2. Prepare a PCR master mix (25 µl or 50 µl) by mixing sterilized DNase-free water, buffer, dNTPs, primers, and Taq DNA Polymerase.
3. Prepare a sufficient master mix according to the number of reactions.
4. Aliquot the master mix into individual PCR tubes kept on ice and add the template DNA.
5. Gently vortex and spin down before
6. Transfer the tubes in a thermocycler preheated at 95°C for amplification as per the desired settings.

Reaction Components	25µl Reaction	50µl Reaction	Final Concentration
10X PCR Reaction Buffer	2.5 µl	5 µl	1X
2.5mM dNTPs	2 µl	4 µl	200µM
10µM Forward Primer	1 µl	1 µl	0.2µM (0.1-0.5µM)
10µM Reverse Primer	1 µl	1 µl	0.2µM (0.1-0.5µM)
Taq DNA polymerase	0.5 µl	1 µl	1.25 - 5 U
Template DNA	1 µl	2 µl	10-100 ng
Nuclease Free Water	17.25 µl	36.5 µl	-

PCR Cycles and Conditions

Initial Denaturation - 95°C - 1 min - 1 Cycle
*Denaturation - 95°C - 30 Sec
*Annealing - 55°C-60°C - 30 Sec
*Extension - 72°C - 1 min
Final extension - 72°C - 5-15 min - 1 Cycle

***25-35 cycles of amplification are recommended.**

Initial denaturation

The initial denaturation step ensures to completely denatures the template DNA by heating it to 94°C or higher for effective utilization of the template. An initial denaturation for 1 to 3 min at 95°C is recommended if the GC content of the template is less than 50%. For GC-rich templates, this step can be extended up to 10 min. If a longer initial denaturation step is required, Taq DNA Polymerase should be added after the initial denaturation step to prevent a decrease in its activity.

Denaturation

A DNA denaturation time of 15 to 30 seconds per cycle at 95°C is generally recommended. In the denaturation step, the two intertwined strands of DNA separate from one another, producing the necessary single-stranded DNA template for replication by the thermostable DNA polymerase.

Annealing

The annealing temperature should be 5°C lower than the melting temperature (Tm) of the primers and is typically 45–68°C. Annealing for 30 to 50 seconds is generally recommended. For non-specific PCR products, annealing temperature can be optimized by temperature gradient PCR with stepwise 1-2°C increments.

Extension

The optimal extension temperature for Taq DNA Polymerase is 70-75°C. The recommended extension step is 1 min at 72°C for PCR products up to 2 kb. For larger products, the extension time should be extended by 1 min/kb. The extension time depends on the length of the amplicon and the complexity of the template. A final extension of 5 minutes at 68°C is recommended.

General guidelines

Template

High-quality, purified DNA templates greatly enhance the success of PCR reactions. Recommended amounts of DNA template for a 50 µl reaction are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 µg for genomic DNA. Higher concentration of template increases the risk of non-specific PCR products whereas lower concentration reduces the accuracy of the amplification. Trace amounts of reagents used for DNA purification, such as phenol, EDTA, and proteinase K, can inhibit DNA polymerases therefore repeated washed of the pellet with 70% ethanol is recommended.

Primers

Primers are generally 20–40 nucleotides in length with a GC content of 40–60%. The recommended final concentration of each primer should be 0.1-1 mM. We recommend, using 0.4 mM as a final concentration (i.e. 20 pmol of each primer per 50 mL reaction volume). Too high a concentration can result in non-specific PCR products. Primers should have a melting temperature (Tm) of approximately 60 °C.

$MgCl_2$

Mg2+ binds to dNTPs, primers, and DNA templates and therefore its concentration needs to be optimized for maximal PCR yield. The recommended Mg2+ concentration range is 1-4 mM.

For standard PCR with Taq DNA Polymerase, the optimal Mg++ concentration is 1.5–2.0 mM. The final Mg++ concentration in 1X Standard Taq Reaction Buffer is 1.5 mM, which is sufficient for the amplification of most amplicons. Mg2+ binds to dNTPs, primers, and DNA templates and therefore its concentration needs to be optimized for maximal PCR yield. If the Mg2+ concentration is too low, PCR product yield will be less whereas with a high Mg2+ concentration non-specific PCR products may appear. If the DNA samples contain EDTA or other metal chelators, the Mg2+ ion concentration should be increased accordingly (1 molecule of EDTA binds one Mg2+).

dNTPs

The final concentration recommended for each dNTPs is 200 µM.

Taq polymerase

We recommend using Taq DNA Polymerase (ProCyto Labs) at a concentration of 5 units/µl reaction. However, the optimal concentration of Taq DNA Polymerase may range from 5–50 units/ml (0.25–5 units/50 µl reaction) according to different applications.