AMPLIQON
PCR ENZYMES & REAGENTS

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MADE IN **DENMARK**

Taq DNA Polymerase RED

Concentration: 5 units/µl

Cat. No.	Taq DNA Polymerase RED ID: 5101750	10x Ammonium Buffer, 15 mM $MgCl_2$ ID: 5100950	10x Standard Buffer, 15 mM $MgCl_2$ ID: 5100510	10x Combination Buffer, 15 mM $MgCl_2$ ID: 5600400	$MgCl_2$ 25 mM ID: 5575801
A206199	20 µl	1.5 ml	1.5 ml	1.5 ml	1.5 ml

Key Features

Ampliqon Taq DNA Polymerase is a thermostable, recombinant DNA polymerase, which exhibits very high activity in primer extension and other molecular biology applications. The enzyme is isolated from *Thermus aquaticus* and has a molecular weight of approximately 94 kDa. Ampliqon Taq DNA Polymerase has a 5'→3' DNA polymerase and a 5'→3' exonuclease activity. The enzyme lacks a 3'→5' exonuclease activity (no proofreading ability).
Taq DNA Polymerase RED contains a red dye which provides easy and quick identification of reactions to which enzyme was added and allows confirmation of complete mixing. The inert dye has no effect on downstream processes. Taq DNA Polymerase RED is added directly to the reaction mix and is used in the same manner as standard Taq DNA Polymerase.

Kit Components

Ampliqon Taq DNA Polymerase RED in Storage Buffer

5 U/µl Taq, 20 mM Tris-HCl pH 8.3, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, inert dye, 0.5% Tween® 20, 50% glycerol.

10x Ammonium Buffer

Tris-HCl pH 8.5, $(NH_4)_2S0_4$, 15 mM $MgCl_2$, 1% Tween® 20.

Ammonium in the buffer minimizes the need for optimization of the $MgCl_2$ concentration or the annealing temperature for most primer-template systems.

10x Standard Buffer

Tris-HCl pH 8.5, KCl, 15 mM $MgCl_2$, 1% Tween® 20.

Standard Buffer is the traditional potassium (K^+) buffer. Standard Buffer promotes high specificity and careful optimization of primer annealing temperatures and Mg^{2+} concentrations may be required.

10x Combination Buffer

Tris-HCl, pH 8.7, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$, 1% Tween® 20.

Combination Buffer is a proprietary mixture of K^+ and NH_4^+. This buffer combines high specificity with good product yield and high tolerance to optimization of primer annealing temperatures and Mg^{2+} concentrations.

25 mM $MgCl_2$

Recommended Storage and Stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.
Option: Store at +4 °C for up to 6 months.

Quality Control

Taq DNA Polymerase is tested for contaminating activities, with no traces of endonuclease activity, nicking activity or exonuclease activity.

Unit Definition

One unit is defined as the amount of polymerase that incorporates 10 nmol of dNTPs into acid-precipitable DNA in 30 minutes at 72 °C under standard assay conditions.

Protocol

This protocol serves as a guideline to ensure optimal PCR results when using Taq DNA Polymerase. Optimal reaction conditions such as incubation times, temperatures and amount of template DNA may vary and must be determined individually.

1. Thaw 10x Buffer, dNTP mix and primer solutions. **It is important to thaw the solutions completely (some buffers need to reach room temperature) and mix thoroughly before use to avoid localized concentrations of salts**. Keep all components on ice. The polymerase is provided in glycerol and does not need thawing. Keep it at -20 °C at all times.

2. Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis. Work on ice at all times.

3. Prepare a master mix according to Table 1. The master mix typically contains all the components needed for extension except the template DNA.

Table 1. Reaction components (master mix and template DNA)

Component	Vol./reaction*	Final concentration*
10x Buffer	5 µl	1x
25 mM $MgCl_2$	0 µl (0 – 6 µl)	1.5 mM (1.5 – 4.5 mM)
dNTP mix (10 mM each)	1 µl	0.2 mM of each dNTP
Primer A (10 µM)	1 µl (0.5 – 5 µl)	0.2 µM (0.1 – 1.0 µM)
Primer B (10 µM)	1 µl (0.5 – 5 µl)	0.2 µM (0.1 – 1.0 µM)
Taq DNA Pol.	0.2 µl (0.2 – 1 µl)	1 unit (1 – 5 units)
PCR-grade H_2O	X µl	-
Template DNA	X µl	genomic DNA: 50 ng (10 – 500 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
TOTAL volume	50 µl	-

* Suggested starting conditions; theoretically used conditions in brackets. The final volume can be reduced to 25 µl by using half of the volumes suggested in Vol./reaction, e.g. 0.1 µl Taq instead of 0.2 µl Taq.

4. Mix the master mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, e.g. by pipetting the master mix up and down a few times.
5. Add template DNA to the individual tubes containing the master mix.
6. Program the thermal cycler according to the manufacturer's instructions.
   For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.
7. Place the tubes in the thermal cycler and start the reaction.

Three-step PCR program

Cycles	Duration of cycle	Temperature
1	2 – 5 minutes[a]	95 °C
25 - 35	20 – 30 seconds[b] 20 – 40 seconds[c] 30 seconds[d]	95 °C 50 – 65 °C 72 °C
1	5 minutes[e]	72 °C

a. Initial denaturation step (optional).

b. Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 20 – 30 seconds. It causes melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

c. Annealing step: The reaction temperature is lowered to 50 – 65 °C for 20 – 40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically, the annealing temperature is about 3 – 5 °C below the T_m (melting temperature) of the primers used.

d. Extension/elongation step: Taq polymerase has its optimum activity temperature at 72 °C. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand. The extension time depends on the length of the DNA fragment to be amplified. As a rule of thumb, at its optimum temperature the DNA polymerase will polymerize a thousand bases per minute.

e. Final elongation: This single step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

Two-step PCR program

Fast 2-step PCR protocols are available using this link:
https://ampliqon.com/en/pcr-technology/application-notes/

Notes:

- 15 mM $MgCl_2$ is present in 10x PCR Buffers. The 1x concentration is 1.5 mM $MgCl_2$. In some applications, more than 1.5 mM $MgCl_2$ is required for best results. For this reason, 25 mM $MgCl_2$ is included in the kit. Table 2 provides the volume of 25 mM $MgCl_2$ to be added to the master mix if a higher $MgCl_2$ concentration is required.

Table 2. Additional volume (µl) of $MgCl_2$ per 50 µl reaction

Final $MgCl_2$ conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Volume of 25 mM $MgCl_2$	0	1.0	2.0	3.0	4.0	5.0	6.0

- For longer DNA targets more DNA polymerase could be added to the PCR master mix.

Other product sizes, combinations and customized solutions are available. Please look at www.ampliqon.com or ask for our complete product list for PCR Enzymes. For customized solutions please contact us.

Made in Denmark

Issued 08/2025