AMPLIQON
PCR ENZYMES & REAGENTS

Taq DNA Polymerase

Concentration: 5 units/µl

Cat. No.: A110003

-	Size Units	Taq DNA Polymerase 5 U/µl
ID No. Cap colour	-	5101600 Purple
A110003	500	100 µl

Key Features

- High activity
- No proofreading – lacks a 3'→5' exonuclease activity
- Ideal for TA cloning – leaves an A' overhang

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 leaves an A' overhang, which makes the enzyme ideal for TA cloning.
We recommend using the Ampliqon Taq DNA Polymerase with one of the Ampliqon Buffers.

Buffers

Ampliqon offers 3 different buffers to allow the customer to choose the optimal buffer system for a specific amplification process. Ampliqon Buffers are usually supplied in 10x formulations with 15 mM $MgCl_2$ included but are also available as Mg^{2+} free buffers, detergent free buffers as well as Mg^{2+} and detergent free buffers.

Ammonium Buffer

Composition: Tris-HCl pH 8.5, $(NH_4)_2SO_4$, 15 mM $MgCl_2$*, 1% Tween® 20*. Ammonium Buffer (NH_4^+) usually gives a superior amplification signal (high yield) in many primer-template systems. Ammonium in the buffer minimizes the need for optimization of the $MgCl_2$ concentration or the annealing temperature for most primer-template systems.

Standard Buffer

Composition: Tris-HCl pH 8.5, KCl, 15 mM $MgCl_2$*, 1% Triton X-100*. 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.

Combination Buffer

Composition: 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 due to its balanced ammonium-potassium formulation.

Magnesium

Mg^{2+} is required for polymerase activity. Low Mg^{2+} concentrations increase the fidelity but with too low Mg^{2+} concentrations the polymerase will not work. The Mg^{2+} concentration available in the reaction is dependent on several parameters e.g. the presence of chelators or the dNTP concentration. Therefore the Mg^{2+} concentration should be optimized.

Tween, Triton

Non-ionic detergents are used to prevent the polymerase to stick to the walls of the tube, to stabilize the polymerase and increase yield. However, these agents might increase non-specific amplification or interfere with downstream reactions. Tween can be used to neutralize SDS contaminations in the DNA template.

Kit Components

Ampliqon Taq DNA Polymerase in Storage Buffer

5 U/µl Taq, 20 mM Tris-HCl pH 8.3, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween® 20, 0.5% NP40, 50% glycerol.

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 trace of endonuclease activity, nicking activity or exonuclease activity.

Unit Definition

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

Protocol

This protocol serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures and amount of template DNA may vary and must be determined individually.

Notes:

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis. Work on ice at all times.
- 15 mM $MgCl_2$ is present in common Ampliqon 10x 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 1 provides the volume of 25 mM $MgCl_2$ to be added to the master mix if a higher $MgCl_2$ concentration is required.

Table 1. 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	2	3	4	5	6

1. Thaw 10x Buffer, dNTP mix and primer solutions.

* optional without Mg^{2+} and/or Tween® 20/Triton X-100

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. Prepare a master mix according to Table 2. The master mix typically contains all the components needed for extension except the template DNA.

Table 2. Reaction components (master mix and template DNA)

Component	Vol./reaction*	Final concentration*
10x Buffer	5 µl	1x
25 mM $MgCl_2$	0 µl (0 – 6.5 µl)	1.5 mM (0.5 – 5 mM)
dNTP mix (12.5 mM each)	0.8 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, eg. 0.1 µl Taq instead of 0.2 µl Taq.

3. 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.
4. Add template DNA to the individual tubes containing the master mix.
5. 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.
6. 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.

Related Products

Taq Polymerase (500 units) *	Cat. No.
Taq DNA Polymerase 5 U/µl	**A110003**
• with 10x Ammonium Buffer	**A111103**
• 5x PCR Buffer RED	**A111803**
Taq DNA Polymerase 5 U/µl, RED	**A200003**
• with 10x Ammonium Buffer	**A201103**
Taq DNA Polymerase 5 U/µl, glycerol free	**A100003**
• with 10x Ammonium Buffer	**A101103**

Hot Start Polymerase (500 units) *	Cat. No.
TEMPase Hot Start DNA Polymerase, 5 U/µl	**A220003**
• with 10x Ammonium Buffer	**A221103**
• 5x PCR Buffer RED	**A221803**
TEMPase Hot Start DNA Polymerase, glycerol free 5 U/µl	**A240003**
• with 10x Ammonium Buffer	**A241103**

High Fidelity - Proof reading (500 units) **	Cat. No.
AccuPOL DNA Polymerase 2.5 U/µl	**A210003**
• with 10x Ammonium Buffer	**A211103**

*Available in kits including one or two buffers (Ammonium Buffer, Standard Buffer or Combination Buffer). **AccuPOL only available in kits with Ammonium Buffer. All kits include extra 25 mM $MgCl_2$.

Buffers for DNA polymerases *	Cat. No.
10x Ammonium Buffer, 3 x 1.5 ml	**A301103**
10x Standard Buffer, 3 x 1.5 ml	**A302103**
10x Combination Buffer, 3 x 1.5 ml	**A303103**
5x PCR Buffer RED, 6 x 1,5 ml **	**A301810**

*Ammonium Buffer, Standard Buffer and Combination Buffer are also available as Mg^{2+} free buffers, detergent free buffers and Mg^{2+} and detergent free buffers. **For direct gel loading and visualisation.

Taq Master Mixes (500 x 50 µl reactions) *	Cat. No.
2x Master Mix, 1.5 mM $MgCl_2$ final concentration	**A140303**
2x Master Mix RED, 1.5 mM $MgCl_2$ final concentration	**A180303**

TEMPase Hot Start Master Mixes (500 x 50 µl reactions) *	Cat. No.
2x Master Mix A**, 1.5 mM $MgCl_2$ final concentration	**A230303**
2x Master Mix A**BLUE, 1.5 mM $MgCl_2$ final concentration	**A290403**

*Master mixes available also in 1.1x variants as well as 2 mM $MgCl_2$ variants, **Mix A is Ammonium Buffer based, also available as Mix C based on Combination Buffer.

Special Master Mixes (500 x 50 µl reactions)	Cat. No.
Multiplex 2x Master Mix, 3 mM $MgCl_2$ final concentration	**A260303**
GC TEMPase 2x Master Mix I – for GC-rich templates	**A331703**
GC TEMPase 2x Master Mix II – for GC-rich templates	**A332703**

Real-time PCR Master Mixes (400 x 25 µl reactions)	Cat. No.
RealQ Plus 2x Master Mix for probe,	
• without ROX™	**A313402**
• with low ROX™	**A314402**
• with high ROX™	**A315402**
RealQ Plus 2x Master Mix Green	
• without ROX™	**A323402**
• with low ROX™	**A324402**
• with high ROX™	**A325402**

Ultrapure dNTPs*	Cat. No.
dNTP Mix 40 mM (2 x 500 µl): 10 mM each dA, dC, dG, dT	**A502004**
dNTP Set, 100 mM each: 250 µl of each dA, dC, dG and dT	**A511104**

*Other concentrations and Single dNTPs are available.

Loading Buffers and Ladders	Cat. No.
5x Loading Buffer Red *, 5 x 1 ml	**A608104**
PCR DNA Ladder **, 100 – 3000 bp, 1 x 0.5 ml	**A610341**

* Also available with Blue, Orange or Cyan. ** Available in different size ranges.

Reagents for *in vitro* laboratory use only.

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 02/2017