AMPLIQON
PCR ENZYMES & REAGENTS

Taq DNA Polymerase, Glycerol Free

Concentration: 5 units/µl

For automation and freeze drying.

Key Features and General Description: Taq Polymerase

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

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.

Ampliqon Taq DNA Polymerase, glycerol free is supplied at a concentration of 5 U/µl in Taq Storage Buffer. The Taq Storage Buffer composition is: 20 mM Tris-HCl pH 8.5, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween® 20, 0.5% NP40.

Storage and Stability

The unopened enzyme is stable at -20 °C for 2 years after the production date.

Quality Control

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

Key Features and General Description: 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.

Suggested Protocol Using Taq DNA Polymerase

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 using a 10x Buffer including 15 mM $MgCl_2$

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

- When using Mg^{2+} free buffers, the addition of $MgCl_2$ to the reaction is imperative because Mg^{2+} is required for polymerase activity. Use 25 mM $MgCl_2$ to adjust the Mg^{2+} concentration according to Table 2.

Table 2. Additional volume (µl) of $MgCl_2$ per 50 µl reaction using a Mg^{2+} free 10x Buffer

Final $MgCl_2$ conc. in reaction (mM)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Volume of 25 mM $MgCl_2$	1	2	3	4	5	6	7	8	9

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**

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

use to avoid localized concentrations of salts. Keep all components on ice.

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

Table 3. Reaction components (master mix and template DNA)

Component	Vol./reaction*	Final concentration*
10x Buffer	5 µl	1x
25 mM $MgCl_2$**	0 µl (0 – 7 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. 5 U/µl	0.6 µl (0.2 –1 µl)	3 units (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
** $MgCl_2$ addition when using 10x Buffers containing 15 mM $MgCl_2$. See table 2 for information when using Mg^{2+} free buffers.

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, recommended when using gDNA).

[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.

Available Products

Taq DNA Polymerase, glycerol free	500 U	1000 U	2500 U	5000 U
	1 tube x 0.1 ml	2 tubes x 0.1 ml	5 tubes x 0.1 ml	10 tubes x 0.1 ml
	Cat. no.	Cat. no.	Cat. no.	Cat. no.
Taq DNA Polymerase without Buffer:				
Taq DNA Polymerase 5 U/µl, without Buffer	A100003	A100004	A100006	A100007
Taq DNA Polymerase with 1 Buffer:				
Taq DNA Polymerase 5 U/µl, 10x Ammonium Buffer (15 mM $MgCl_2$) and $MgCl_2$	A101103	A101104	A101106	A101107
Taq DNA Polymerase 5 U/µl, 10x Standard Buffer (15 mM $MgCl_2$) and $MgCl_2$	A102103	A102104	A102106	A102107
Taq DNA Polymerase 5 U/µl, 10x Combination Buffer (15 mM $MgCl_2$) and $MgCl_2$	A103103	A103104	A103106	A103107
Taq DNA Polymerase 5 U/µl, 10x Ammonium Buffer (Mg^{2+} free) and $MgCl_2$	A101203	A101204	A101206	A101207
Taq DNA Polymerase 5 U/µl, 10x Standard Buffer (Mg^{2+} free) and $MgCl_2$	A102203	A102204	A102206	A102207
Taq DNA Polymerase 5 U/µl, 10x Combination Buffer (Mg^{2+} free) and $MgCl_2$	A103203	A103204	A103206	A103207
Taq DNA Polymerase with 2 Buffers:				
Taq DNA Polymerase 5 U/µl, 10x Ammonium Buffer (15 mM $MgCl_2$), 10x Standard Buffer (15 mM $MgCl_2$) and $MgCl_2$	A104103	A104104	A104106	A104107
Taq DNA Polymerase 5 U/µl, 10x Ammonium Buffer (15 mM $MgCl_2$), 10x Combination Buffer (15 mM $MgCl_2$) and $MgCl_2$	A105103	A105104	A105106	A105107
Taq DNA Polymerase 5 U/µl, 10x Ammonium Buffer (Mg^{2+} free), 10x Standard Buffer (Mg^{2+} free) and $MgCl_2$	A104203	A104204	A104206	A104207
Taq DNA Polymerase 5 U/µl, 10x Ammonium Buffer (Mg^{2+} free), 10x Combination Buffer (Mg^{2+} free) and $MgCl_2$	A105203	A105204	A105206	A105207

Buffers are also available as detergent free buffers and Mg^{2+} and detergent free buffers.

Other product sizes, combinations and customized solutions are available.
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