AMPLIQON
PCR ENZYMES & REAGENTS

TEMPase Hot Start DNA Polymerase

Concentration: 5 units/µl

For reaction setup at room temperature, superior amplification and high specificity. Suitable for detection of low copy number targets and for multiplex PCR.

Key Features and General Description: TEMPase

- TEMPase Hot Start enzyme for increased specificity, sensitivity and product yield
- Successful multiplex reactions save time and reagents
- Designed to diminish the formation of non-specific product
- Detection of low target copy number

TEMPase Hot Start DNA Polymerase is a modified form of Ampliqon Taq DNA Polymerase, which features higher specificity, superior sensitivity and greater yields when compared to standard DNA polymerases.
TEMPase Hot Start DNA Polymerase is activated by heat treatment. A chemical moiety is attached to the enzyme at the active site, which renders the enzyme inactive at room temperature. Thus, during setup and the first ramp of thermal cycling, the enzyme is not active and misprimed primers are not extended. Once the reaction reaches optimal activating temperature, the chemical moiety is cleaved during a 15 minutes heat activation step, releasing the active TEMPase Hot Start DNA Polymerase into the reaction.

Ampliqon TEMPase Hot Start DNA Polymerase is supplied at a concentration of 5 U/µl in TEMPase Storage Buffer. The TEMPase Storage Buffer composition is: 20 mM Tris-HCl pH 8.9, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween® 20, 0.5% NP40, 50% glycerol.

Storage and Stability

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

Quality Control

TEMPase Hot Start 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 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^+) 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 TEMPase Hot Start 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. Using TEMPase Hot Start DNA Polymerase, working on ice is not needed.
- 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 use to avoid localized concentrations of salts**. The polymerase is provided in glycerol and does not need thawing.

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)
TEMPase DNA Pol.	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.

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

5. Program the thermal cycler according to the manufacturer's instructions. **Each program must start with an initial heat activation step at 95°C for 15 minutes.**
   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

[a] Cycles	Duration of cycle	Temperature
1	15 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

[b] For activation of the TEMPase hot start enzyme.

[c] 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.

[d] 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.

[e] Extension/elongation step: TEMPase 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.

[f] 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

TEMPase Hot Start DNA Polymerase	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.
TEMPase DNA Polymerase without Buffer:				
TEMPase DNA Polymerase 5 U/µl, without Buffer	A220003	A220004	A220006	A220007
TEMPase DNA Polymerase with 1 Buffer:				
TEMPase DNA Polymerase 5 U/µl, 10x Ammonium Buffer (15 mM $MgCl_2$) and $MgCl_2$	A221103	A221104	A221106	A221107
TEMPase DNA Polymerase 5 U/µl, 10x Combination Buffer (15 mM $MgCl_2$) and $MgCl_2$	A223103	A223104	A223106	A223107
TEMPase DNA Polymerase 5 U/µl, 10x Ammonium Buffer (Mg^{2+} free) and $MgCl_2$	A221203	A221204	A221206	A221207
TEMPase DNA Polymerase 5 U/µl, 10x Combination Buffer (Mg^{2+} free) and $MgCl_2$	A223203	A223204	A223206	A223207
TEMPase DNA Polymerase with 2 Buffers:				
TEMPase DNA Polymerase 5 U/µl, 10x Ammonium Buffer (15 mM $MgCl_2$), 10x Combination Buffer (15 mM $MgCl_2$) and $MgCl_2$	A225103	A225104	A225106	A225107
TEMPase DNA Polymerase 5 U/µl, 10x Ammonium Buffer (Mg^{2+} free), 10x Combination Buffer (Mg^{2+} free) and $MgCl_2$	A225203	A225204	A225206	A225207

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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