AMPLIQON
PCR ENZYMES & REAGENTS

15 MIN HOT START

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TEMPase Hot Start DNA Polymerase Glycerol Free

Concentration: 5 units/µl

MADE IN **DENMARK**

Cat. No.	TEMPase Hot Start DNA Pol. Glycerol free ID: 5101700	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	5x PCR Buffer RED 7.5 mM $MgCl_2$ ID: 5100100	$MgCl_2$ 25 mM ID: 5575801
A246199	20 µl	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml

Key Features

TEMPase Hot Start DNA Polymerase, Glycerol Free, is a high-quality DNA polymerase developed for automation. It is a glycerol free formulation of TEMPase Hot Start Polymerase with the same excellent performance. The glycerol free formulation makes it well suited for automation and freeze-drying.

TEMPase Hot Start DNA Polymerase is a modified form of Ampliqon Taq DNA Polymerase. 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-minute heat activation step, releasing the active TEMPase Hot Start DNA Polymerase into the reaction.

Kit Components

TEMPase Hot Start DNA Polymerase in Glycerol Free Storage Buffer

5 U/µl TEMPase Hot Start DNA Polymerase, 20 mM Tris-HCl pH 8.9, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween® 20.

10x Ammonium Buffer

Tris-HCl pH 8.5, $(NH_4)_2SO_4$, 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.

5x PCR Buffer RED

Tris-HCl pH 8.5, $(NH_4)_2SO_4$, 7.5 mM $MgCl_2$, 0.5% Tween® 20, red tracking dye, density agent.

$MgCl_2$

25 mM $MgCl_2$ in PCR grade water.

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 1 month.

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 Solutions. **It is important to thaw all 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.

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 2. The master mix typically contains all the components needed for extension except the template DNA.

Table 2. Reaction mix and template DNA

Component		Vol./reaction*	Final concentration*
10x Buffer 5x Buffer	*or*	5 µl 10 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)
TEMPase DNA Pol.		0.4 µl (0.2 – 1 µl)	2 units (1 – 5 units)
PCR-grade H_2O		X µl	-
Template DNA		X µl	genomic DNA: 20 ng (1 – 200 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. 0.2 µl TEMPase instead of 0.4 µl TEMPase.

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. **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 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	15 minutes[a]	95 °C
25 – 35	20 – 30 seconds[b] 20 – 40 seconds[c] 30 – 90 seconds[d]	95 °C 50 – 65 °C 72 °C
1	5 minutes[e]	72 °C

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

[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: 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.

[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.

Notes:

- 15 mM $MgCl_2$ is present in 10x PCR Buffer (7.5 mM is present in the 5x PCR Buffer RED). 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.

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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

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