AMPLIQON
PCR ENZYMES & REAGENTS

15 MIN HOT START

Ampliqon A/S
www.ampliqon.com
info@ampliqon.com

Stenhuggervej 22
5230 Odense M
Denmark

Phone: +45 70201169
VAT No.: DK 26388899

TEMPase Hot Start DNA Polymerase

Concentration: 5 units/µl

MADE IN **DENMARK**

Cat. No.	Units	TEMPase Hot Start DNA Pol. ID: 5101650	10x Ammonium Buffer, 15 mM $MgCl_2$ ID: 5100950	$MgCl_2$ 25 mM ID: 5575801
A221102	250	50 µl	1.5 ml	1.5 ml
A221103	500	100 µl	1.5 ml	1.5 ml
A221104	1000	2 x 100 µl	2 x 1.5 ml	2 x 1.5 ml
A221106	2500	5 x 100 µl	5 x 1.5 ml	5 x 1.5 ml
A221107	5000	10 x 100 µl	3 x 5 ml	3 x 5 ml
A221108	10,000	3 x 667 µl	6 x 5 ml	6 x 5 ml

Key Features

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 minutes heat activation step, releasing the active TEMPase Hot Start DNA Polymerase into the reaction.

Kit Components

TEMPase Hot Start DNA Polymerase in 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, 50% glycerol.

10x Ammonium Buffer

Tris-HCl pH 8.5, $(NH_4)_2SO_4$, 15 mM $MgCl_2$, 1% Tween® 20.

Ammonium Buffer gives a superior amplification signal (high yield) in most primer-template systems. Ammonium in the buffer minimizes the need for optimization of the $MgCl_2$ concentration and the annealing temperature.

$MgCl_2$

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 6 months.

Quality Control

TEMPase Hot Start 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 TEMPase Hot Start 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**. The polymerase is provided in glycerol and does not need thawing.

   Important: Spin vials briefly before use.

2. Prepare a reaction mix according to table 1. The reaction mix typically contains all the components needed for primer extension except the template DNA.

Table 1. Reaction 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)
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, eg. 0.2 µl TEMPase instead of 0.4 µl TEMPase.

3. Mix the reaction mix thoroughly and dispense appropriate volumes into reaction tubes.

4. Add template DNA to the individual tubes containing the reaction 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 or primer pair.

6. Place the tubes in the thermal cycler and start the reaction.

Table 3. 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.

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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. 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 3 provides the volume of 25 mM $MgCl_2$ to be added to the master mix if a higher $MgCl_2$ concentration is required.

Table 3. 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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Made in Denmark

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