AMPLIQON
PCR ENZYMES & REAGENTS

TEMPase Hot Start DNA Polymerase, Glycerol Free

With 10x Ammonium Buffer (15 mM $MgCl_2$)

Concentration: 5 units/µl

Cat. No.: A241104

-	Size Units	TEMPase, Glycerol Free, 5 U/µl	10x Ammonium Buffer, 15 mM $MgCl_2$	$MgCl_2$ 25 mM
ID No. Cap colour	-	**5101700 White**	**5100950 White***	**5575801 Yellow***
A241102	250	50 µl	1.5 ml	1.5 ml
A241103	500	100 µl	1.5 ml	1.5 ml
A241104	1000	2 x 100 µl	2 x 1.5 ml	2 x 1.5 ml
A241106	2500	5 x 100 µl	5 x 1.5 ml	5 x 1.5 ml
A241107	5000	10 x 100 µl	3 x 5 ml	3 x 5 ml
A241108	10000	3 x 667 µl	6 x 5 ml	6 x 5 ml

* 5 ml tubes have clear caps

Key Features

- Convenient reaction set-up at room temperature
- Increased specificity, sensitivity and product yield
- Detection of low copy number targets
- Diminished formation of non-specific product
- For automation and freeze-drying

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 10 – 15 minutes heat activation step, releasing the active TEMPase Hot Start DNA Polymerase into the reaction.

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.

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, 0.5% NP40.

10x Ammonium Buffer

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

$MgCl_2$

25 mM $MgCl_2$ in PCR grade water.

Storage

The unopened kit is stable at -20 °C for: Not determined.

Quality Control

TEMPase Hot Start DNA Polymerase is tested for contaminating activities, with no traces 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.

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. Working on ice is not required.
- 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 reaction mix if a higher $MgCl_2$ concentration than 1.5 mM 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. **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**.

   Important: Spin vials briefly before use.

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

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.

Table 2. Reaction 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 (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

6. 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.

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The used Hot Start technology is patented in the following countries; Austria, Finland, France, Germany, Great Britain, Italy, Japan, Spain, Sweden, Switzerland and USA. A Hot Start license for use in research in these countries is included with this product, therefore the notice below.

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Related Products

Taq Polymerase Kits (500 units)	Cat. No.
Taq DNA Polymerase 5 U/µl	**A110003**
• with 10x Ammonium Buffer	**A111103**
• with 10x Standard Buffer	**A112103**
• with 10x Ammonium Buffer and 10x Standard Buffer	**A114103**
Taq DNA Polymerase 5 U/µl, RED	**A200003**
• with 10x Ammonium Buffer	**A201103**
• with 10x Standard Buffer	**A202103**
Taq DNA Polymerase 5 U/µl, glycerol free	**A100003**

Hot Start Polymerase (500 units)	Cat. No.
TEMPase Hot Start DNA Polymerase, 5 U/µl	**A220003**
• with 10x Ammonium Buffer	**A221103**
• with 10x Standard Buffer	**A222103**
• with 4 x GC Buffer I and 4 x GC Buffer II	**A227103**

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

All polymerases are also available in kits including one or two buffers (with 15 mM $MgCl_2$, Mg^{2+} free, detergent free or Mg^{2+} and detergent free) and 25 mM $MgCl_2$.

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 C, 1.5 mM $MgCl_2$ final concentration	**A230703**
2x Master Mix A BLUE, 1.5 mM $MgCl_2$ final concentration	**A290403**
2x Master Mix C BLUE, 1.5 mM $MgCl_2$ final concentration	**A290803**

* Mix A is Ammonium Buffer based, Mix C is Combination Buffer based

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 (400 x 25 µl reactions)	Cat. No.
RealQ Plus 2x Master Mix for probe, without ROX™	**A313402**
RealQ Plus 2x Master Mix for probe, low ROX™	**A314402**
RealQ Plus 2x Master Mix for probe, high ROX™	**A315402**
RealQ Plus 2x Master Mix Green, without ROX™	**A323402**
RealQ Plus 2x Master Mix Green, low ROX™	**A324402**
RealQ Plus 2x Master Mix Green, 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.

Buffers, Loading Buffers and Ladders	Cat. No.
25mM $MgCl_2$, 3 × 1.5 ml	**A308103**
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 Loading Buffer Red, 5 x 1 ml	**A608104**
PCR DNA Ladder, 100 – 3000 bp, 1 x 0.5 ml	**A610341**

Buffers are also available as Mg^{2+} free buffers, detergent free buffers and Mg^{2+} and detergent free buffers. Other product sizes, combinations and customized solutions are available.

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