AMPLIQON
PCR ENZYMES & REAGENTS

GC TEMPase 2x Master Mix I

Cat. No.: A331706

Cat. No.	Size Reactions	GC TEMPase 2x Master Mix I, 1.5 mM $MgCl_2$
ID No. Cap colour	-	5300400 Yellow
A331799	20	0.5 ml
A331701	100	2 x 1.25 ml
A331703	500	10 x 1.25 ml
A331704	1000	20 x 1.25 ml
A331706	2500	50 x 1.25 ml
A331707	5000	25 x 5 ml

* 5 ml tubes have clear caps

Key Features

- For amplification of DNA targets with high GC content
- Convenient reaction set-up at room temperature
- High specificity, sensitivity and product yield
- Detection of low abundance targets
- Diminished formation of non-specific product

GC TEMPase 2x Master Mix I is an all-in-one 2x master mix containing TEMPase Hot Start DNA polymerase, GC Buffer I, enhancer, dNTPs and $MgCl_2$. Simply mix GC TEMPase 2x Master Mix I with primers, template and water and you are ready to carry out successful primer extensions.

TEMPase Hot Start DNA Polymerase is a modified form of Ampliqon Taq DNA polymerase, which 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. The result is higher specificity, increased sensitivity and greater yields when compared to standard DNA polymerases.

Composition of GC TEMPase 2x Master Mix I

- TEMPase Hot Start DNA Polymerase
- Optimized buffer components, 3.0 mM $MgCl_2$
- dNTPs
- Enhancer

Storage and Stability

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

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.

- The final $MgCl_2$ concentration of this Master Mix is 1.5 mM. In some applications, more than 1.5 mM $MgCl_2$ is required for best results. Use 25 mM $MgCl_2$ (see Related Products) to adjust the Mg^{2+} concentration according to table 1.

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 the Master Mix and primer solutions. **It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts**.

   Important: Spin vials briefly before use.

2. Prepare the reaction mix. Table 2 shows the reaction set up for a final volume of 50 µl. If desired, the reaction size may be scaled down.

Table 2. Reaction mix and template DNA

Component	Vol./reaction*	Final concentration*
2x Master Mix	25 µl	1x
25 mM $MgCl_2$	0 µl (0 – 7 µl)	1.5 mM (0.5 – 5 mM)
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)
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

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.** See Table 3 for an example.

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.

Table 3. Three-step PCR program

Cycles	Duration of cycle	Temperature
1	15 minutes[a]	95 °C
25 – 35	30 seconds[b] 40 – 60 seconds[c] 40 – 60 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.

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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