AMPLIQON
PCR ENZYMES & REAGENTS

GC TEMPase 2x Master Mix II

Cat. No.: A332706

A332706

Cat. No.	Size Reactions	GC TEMPase 2x Master Mix II, 1.5 mM $MgCl_2$
ID No. Cap colour	-	5300500 White
A332706	2500	50 x 1.25 ml

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 II is an all-in-one 2x master mix containing TEMPase Hot Start DNA polymerase, GC Buffer II, 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 II

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

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, 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 $MgCl_2$ concentration in the final reaction is 1.5 mM with this Master Mix. 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 $MgCl_2$ 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. Set up each reaction. Table 2 shows the reaction mixmset up for a final volume of 50 µl. If desired, the reaction size may be scaled down.
3.

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

4. Mix the reaction mix thoroughly and dispense appropriate volumes into reaction tubes.
5. Add template DNA to the individual tubes containing the reaction 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.** 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.
7. 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.

NOTICE TO PURCHASER: LIMITED USE OF HOT START LICENSE
The use of this product for research purposes is covered by a license from QIAGEN Group. No rights to use this product to perform or offer diagnostic, companion diagnostic, commercial testing or other commercial services for money or money's worth are granted by the supply of this product expressly, by implication or by estoppel.

Should you wish to use this product for any other purpose not covered by this license, please contact QIAGEN Corporate Business Development at bd@qiagen.com.

Related Products

Taq Polymerase (500 units) *	Cat. No.
Taq DNA Polymerase 5 U/µl	**A110003**
• with 10x Ammonium Buffer	**A111103**
• 5x PCR Buffer RED	**A111803**
Taq DNA Polymerase 5 U/µl, RED	**A200003**
• with 10x Ammonium Buffer	**A201103**
Taq DNA Polymerase 5 U/µl, glycerol free	**A100003**
• with 10x Ammonium Buffer	**A101103**

Hot Start Polymerase (500 units) *	Cat. No.
TEMPase Hot Start DNA Polymerase, 5 U/µl	**A220003**
• with 10x Ammonium Buffer	**A221103**
• 5x PCR Buffer RED	**A221803**
TEMPase Hot Start DNA Polymerase, glycerol free 5 U/µl	**A240003**
• with 10x Ammonium Buffer	**A241103**

High Fidelity - Proof reading (500 units) **	Cat. No.
AccuPOL DNA Polymerase 2.5 U/µl	**A210003**
• with 10x Ammonium Buffer	**A211103**

*Available in kits including one or two buffers (Ammonium Buffer, Standard Buffer or Combination Buffer). **AccuPOL only available in kits with Ammonium Buffer. All kits include extra 25 mM $MgCl_2$.

Buffers for DNA polymerases *	Cat. No.
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 PCR Buffer RED, 6 x 1,5 ml **	**A301810**

*Ammonium Buffer, Standard Buffer and Combination Buffer are also available as Mg^{2+} free buffers, detergent free buffers and Mg^{2+} and detergent free buffers. **For direct gel loading and visualisation.

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 A**BLUE, 1.5 mM $MgCl_2$ final concentration	**A290403**

*Master mixes available also in 1.1x variants as well as 2 mM $MgCl_2$ variants, **Mix A is Ammonium Buffer based, also available as Mix C based on Combination Buffer.

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

Loading Buffers and Ladders	Cat. No.
5x Loading Buffer Red *, 5 x 1 ml	**A608104**
PCR DNA Ladder **, 100 – 3000 bp, 1 x 0.5 ml	**A610341**

* Also available with Blue, Orange or Cyan. ** Available in different size ranges.

Reagents for *in vitro* laboratory use only.

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
Issued 03/2017

Ampliqon A/S, Stenhuggervej 22, DK-5230 Odense M, Denmark. Phone: +45 70201169 Fax: +45 70201179 info@ampliqon.com www.ampliqon.com