AMPLIQON
PCR ENZYMES & REAGENTS

15 MIN HOT START

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GC TEMPase 2x Master Mix II

MADE IN DENMARK

Cat. No.	Reactions (50 µl)	GC TEMPase 2x Master Mix II, 1.5 mM $MgCl_2$ ID: 5300500
A332701	100	2 x 1.25 ml
A332703	500	10 x 1.25 ml
A332704	1000	20 x 1.25 ml
A332706	2500	50 x 1.25 ml
A332707	5000	25 x 5 ml
A332799	Sample - 20	0.5 ml

Key Features

Ampliqon offers a product series specifically developed for the amplification of GC-rich DNA sequences. GC TEMPase Master Mix II promotes excellent and robust amplification results with targets of varying degree of GC content and is especially intended for amplification of DNA targets with high GC content.

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 II 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 yield 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 or exonuclease activity.

Protocol

This protocol serves as a guideline to ensure optimal PCR results when using GC TEMPase 2x Master Mix II. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

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

Table 1. Reaction mix and template DNA

Component	Vol./reaction*	Final concentration*
2x Master Mix	25 µl	1x
25 mM $MgCl_2$	0 µl (0 – 6 µl)	1.5 mM (1.5 – 4.5 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 2 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.

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Notes:

- 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 $MgCl_2$ to adjust the Mg^{2+} concentration according to table 3.

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	2	3	4	5	6

- For longer DNA targets: More DNA polymerase could be added to the PCR master mix.

For Research Use Only. Not for use in diagnostics procedures.

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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