



Multiplex TEMPase 2x Master Mix

3 mM MgCl₂ final concentration

MADE IN DENMARK

Cat. No.: A260399 – SAMPLE

20 Reactions

-	Multiplex TEMPase 2x Master Mix	MgCl ₂ 25 mM
ID No.	5500100	5575801
Cap colour	Green	Clear
Content	0.5 ml	1x 1.5 ml

Key Features

Multiplex PCR is a method that enables amplification of two or more amplicons simultaneously in a single reaction tube. It is widely used in genotyping and different areas of DNA testing in research, forensic and diagnostic laboratories.

Multiplex TEMPase 2x Master Mix facilitates the amplification of multiple PCR products, minimizes the need for optimization, making the development of multiplex PCR assays both fast and simple.

Multiplex TEMPase 2x Master Mix is an all-in-one 2x master mix with TEMPase Hot Start DNA polymerase, optimized buffer system, dNTPs and MgCl₂. Each reaction requires 25 µl of the 2x Master Mix. Simply add primers, template and water to a total reaction volume of 50 µl to successfully carry out multiplex PCR.

TEMPase 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, making this enzyme especially well-suited for multiplex PCR.

Kit Components

Multiplex TEMPase 2x Master Mix

TEMPase Hot Start DNA polymerase, optimized buffer system, dNTPs and 6 mM mMgCl₂

MgCl₂

25 mM MgCl₂ 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.

Protocol

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

1. Thaw Master Mix and primers. **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 mix set-up for one reaction with a final volume of 50 µl.
3. Mix the reaction mix thoroughly and dispense appropriate volumes into reaction tubes.

Table 1. Reaction mix and template DNA

Component	Vol./reaction*	Final concentration*
Master Mix	25 µl	1x
25 mM MgCl ₂	0 µl (0 – 4 µl)	3 mM (3 – 5 mM)
each forward Primer (10 µM)	1 µl (0.5 – 5 µl)	0.2 µM (0.1 – 1.0 µM)
Each reverse primer (10 µM)	1 µl (0.5 – 5 µl)	0.2 µM (0.1 – 1.0 µM)
PCR-grade H ₂ O	X µl	-
Template DNA	X µl	genomic DNA: 200 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. 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 or primer pair.
6. Place the tubes in the thermal cycler and start the reaction.

Table 2. Three-step PCR program

Cycles	Duration of cycle	Temperature
1	15 minutes ^a	95 °C
30 – 40	20 – 30 seconds ^b 50 – 90 seconds ^c 50 – 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.

Notes:

- All primers should have the same melting temperature.
- Use equal concentrations (0.2 µM) of all primers for the initial test.
- If one product gives a much stronger band than the others, reduce the primer concentration for this target.
- If one product gives a much weaker band than the others, increase the primer concentration for this target.
- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis. Working on ice is not required.
- The MgCl₂ concentration in the final reaction is 3 mM with this Master Mix. In some applications, more MgCl₂ is required for best results. Use 25 mM MgCl₂ to adjust the MgCl₂ concentration according to table 3.

Table 3. Additional volume (µl) of MgCl₂ per 50 µl reaction:

Final MgCl ₂ conc. in reaction (mM)	3.0	3.5	4.0	4.5	5.0
Volume of 25 mM MgCl ₂	0	1	2	3	4

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

Related Products

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 MgCl₂ variants, **Mix A is Ammonium Buffer based, also available as Mix C based on Combination Buffer.

Special TEMPase 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

Taq Master Mixes (500 x 50 µl reactions)	Cat. No.
2x Master Mix, 1.5 mM MgCl ₂ final concentration	A140303
2x Taq OptiMix CLEAR, 1.5 mM MgCl ₂ final concentration	A370503
2x Master Mix RED, 1.5 mM MgCl ₂ final concentration	A180303

Taq DNA Polymerase (500 units) *	Cat. No.
Taq DNA Polymerase 5 U/µl	A110003
• with 10x Ammonium Buffer	A111103

*Available in kits including one or two buffers (Ammonium Buffer, Standard Buffer or Combination Buffer). All kits include extra 25 mM MgCl₂

Hot Start DNA Polymerase (500 units) *	Cat. No.
TEMPase Hot Start DNA Polymerase, 5 U/µl	A220003
• with 10x Ammonium Buffer	A221103

*Available in kits including one or two buffers (Ammonium Buffer, Standard Buffer or Combination Buffer). All kits include extra 25 mM MgCl₂

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
PCR Grade Water, 6 x 5 ml	A360056

*Ammonium Buffer, Standard Buffer and Combination Buffer are also available as Mg²⁺ free buffers, detergent free buffers and Mg²⁺ and detergent free buffers.
**For direct gel loading and visualisation.

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

Issued 08/2021