AMPLIQON
PCR ENZYMES & REAGENTS

15 MIN HOT START

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Multiplex TEMPase 2x Master Mix

MADE IN DENMARK

Cat. No.	Reactions (50 µl)	Multiplex TEMPase 2x Master Mix ID: 5500100	$MgCl_2$ 25 mM ID: 5575801
A260301	100	2 x 1.25 ml	1 x 1.5 ml
A260303	500	10 x 1.25 ml	1 x 1.5 ml
A260306	2500	50 x 1.25 ml	3 x 1.5 ml
A260307	5000	25 x 5 ml	2 x 5 ml
A260399	Sample - 20	0.5 ml	1 x 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 $MgCl_2$. 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.

Kit Components

Multiplex TEMPase 2x Master Mix

TEMPase Hot Start DNA polymerase, optimized buffer system, dNTPs and 6 mM $mMgCl_2$

$MgCl_2$

25 mM $MgCl_2$ 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 setup 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_2O	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.** Example in table 2. 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 $MgCl_2$ concentration in the final reaction is 3 mM with this Master Mix. In some applications, more $MgCl_2$ is required for best results. Use 25 mM $MgCl_2$ to adjust the $MgCl_2$ concentration according to table 3.

Table 3. Additional volume (µl) of $MgCl_2$ per 50 µl reaction:

Final $MgCl_2$ conc. in reaction (mM)	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.

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 08/2025