AMPLIQON
PCR ENZYMES & REAGENTS

TEMPase Hot Start 2x Master Mix C

Combination Buffer based, 1.5 mM $MgCl_2$ final concentration

For reaction setup at room temperature, superior amplification and high specificity. Recommended for detection of low copy number targets and for multiplex PCR.

Key Features and General Description

- TEMPase Hot Start enzyme for increased specificity and product yield
- Designed to diminish the formation of non-specific product
- Detection of low copy number targets
- Significantly reduced set up time
- Diminished risk of contamination
- Increased reproducibility

TEMPase Hot Start 2x Master Mixes are ready-to-use 2x reaction mixes with the TEMPase Hot Start DNA polymerase, buffer, dNTPs and magnesium chloride present. 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 primer extensions and other molecular biology applications.

TEMPase Hot Start DNA Polymerase

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 and greater yields when compared to standard DNA polymerases.

Master Mix A: Ammonium Buffer

Ammonium Buffer (NH_4^+) gives a superior amplification signal (high yield) in many primer-template systems. Ammonium in the buffer minimizes the need for optimization of the $MgCl_2$ concentration or the annealing temperature for most primer-template systems.

Master Mix C: Combination Buffer

Combination Buffer is a proprietary mixture of K^+ and NH_4^+. This buffer combines high specificity with good product yield and high tolerance to optimization of primer annealing temperatures and Mg^{2+} concentrations due to its balanced ammonium-potassium formulation.

Composition of 2x TEMPase Hot Start Master Mix C

- Tris-HCl pH 8.7, balanced KCl/$(NH_4)_2SO_4$, 3 mM $MgCl_2$, 0.2% Tween® 20
- 0.4 mM of each dNTP
- 0.2 units/µl TEMPase Hot Start DNA Polymerase

Storage and Stability

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

Quality Control

TEMPase Hot Start DNA Polymerase is tested for contaminating activities, with no trace 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.

Suggested Protocol Using TEMPase Hot Start 2x Master Mixes

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 needed.
- The final $MgCl_2$ concentration of this 2x TEMPase Master Mixes is 1.5 mM. In some applications, more than 1.5 mM $MgCl_2$ is required for best results. Use 25 mM $MgCl_2$ (may be purchased separately) 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 TEMPase 2x Master Mix and primers. **It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts**.
2. Set up each reaction. Table 2 shows the reaction set up for a final volume of 50 µL. If desired, the reaction size may be scaled down. Use 10 µl of the TEMPase 2x Master Mix in a final volume of 20 µl.

Table 2. Reaction components (reaction mix and template DNA)

Component	Vol./reaction*	Final concentration*
TEMPase 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. 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	20 – 30 seconds[b] 20 – 40 seconds[c] 30 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.

[a.] 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.

Related Products

Hot Start: **TEMPase DNA Polymerase 2x Master Mix**	100 R* 2 tubes x 1.25 ml Cat. no.	500 R* 10 tubes x 1.25 ml Cat. no.	2500 R* 50 tubes x 1.25 ml Cat. no.	5000 R* 25 tubes x 5 ml Cat. no.
TEMPase Hot Start 2x Master Mix				
TEMPase Hot Start 2x Master Mix A, based on Ammonium Buffer 1.5 mM $MgCl_2$ (final concentration)	A230301	A230303	A230306	A230307
TEMPase Hot Start 2x Master Mix C, based on Combination Buffer 1.5 mM $MgCl_2$ (final concentration)	A230701	A230703	A230706	A230707
TEMPase Hot Start Master Mix BLUE - for direct loading				
TEMPase Hot Start 2x Master Mix A BLUE, based on Ammonium Buffer 1.5 mM $MgCl_2$ (final concentration)	A290401	A290403	A290406	A290407
TEMPase Hot Start 2x Master Mix C BLUE, based on Combination Buffer 1.5 mM $MgCl_2$ (final concentration)	A290801	A290803	A290806	A290807

* Final reaction volume: 50 µl. Other product sizes, combinations and customized solutions are available.

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