

## TEMPase Hot Start 2x Master Mix A

Ammonium Buffer Based, 1.5 mM MgCl<sub>2</sub> final concentration

MADE IN DENMARK

Cat. No.: A230399 – SAMPLE  
20 Reactions

-	TEMPase Hot Start 2x Master Mix A, Ammonium Buffer Based, 1.5 mM MgCl <sub>2</sub>
ID No.	5200400
Cap colour	Red
A230399	0.5 ml

### Key Features

TEMPase Hot Start 2x Master Mix A is an all-in-one 2x master mix containing TEMPase Hot Start DNA polymerase, the ammonium buffer system, dNTPs and magnesium chloride. 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.

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 2x TEMPase Hot Start Master Mix A

- Tris-HCl pH 8.5, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 3.0 mM MgCl<sub>2</sub>, 0.2% Tween® 20
- 0.4 mM of each dNTP
- TEMPase Hot Start DNA Polymerase
- Stabilizer

### 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

Taq 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 TEMPase Hot Start 2x Master Mix A. 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 mix set up for a final volume of 50 µl.

**Table 1. Reaction mix and template DNA**

Component	Vol./reaction*	Final concentration*
Master Mix	25 µl	1x
25 mM MgCl <sub>2</sub>	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 <sub>2</sub> O	X µl	-
Template DNA	X µl	genomic DNA: 20 ng (1 – 200 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
<b>TOTAL volume</b>	<b>50 µl</b>	-

\* 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 target 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 <sup>a</sup>	95 °C
25 – 35	20 – 30 seconds <sup>b</sup> 20 – 40 seconds <sup>c</sup> 30 – 90 seconds <sup>d</sup>	95 °C 50 – 65 °C 72 °C
1	5 minutes <sup>e</sup>	72 °C

<sup>a</sup> For activation of the TEMPase hot start enzyme.

<sup>b</sup> 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.

<sup>c</sup> 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<sub>m</sub> (melting temperature) of the primers used.

<sup>d</sup> 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.

- <sup>e</sup> 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:**

- The final MgCl<sub>2</sub> concentration of this TEMPase Hot Start 2x Master Mix A is 1.5 mM. In some applications, more than 1.5 mM MgCl<sub>2</sub> is required for best results. Use 25 mM to adjust the Mg<sup>2+</sup> concentration according to table 3.

**Table 3. Additional volume (µl) of MgCl<sub>2</sub> per 50 µl reaction:**

Final MgCl <sub>2</sub> conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Volume of 25 mM MgCl <sub>2</sub>	0	1	2	3	4	5	6

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

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**Related Products**

TEMPase Hot Start Master Mixes (500 x 50 µl reactions) *	Cat. No.
2x Master Mix A**, 1.5 mM MgCl <sub>2</sub> final concentration	<b>A230303</b>
2x Master Mix A**BLUE, 1.5 mM MgCl <sub>2</sub> final concentration	<b>A290403</b>

\*Master mixes available also in 1.1x variants as well as 2 mM MgCl<sub>2</sub> 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 <sub>2</sub> final concentration	<b>A260303</b>
GC TEMPase 2x Master Mix I – for GC-rich templates	<b>A331703</b>
GC TEMPase 2x Master Mix II – for GC-rich templates	<b>A332703</b>

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

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

\*Available in kits including one or two buffers (Ammonium Buffer, Standard Buffer or Combination Buffer). All kits include extra 25 mM MgCl<sub>2</sub>

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

\*Available in kits including one or two buffers (Ammonium Buffer, Standard Buffer or Combination Buffer). All kits include extra 25 mM MgCl<sub>2</sub>

Buffers for DNA polymerases *	Cat. No.
10x Ammonium Buffer, 3 x 1.5 ml	<b>A301103</b>
10x Standard Buffer, 3 x 1.5 ml	<b>A302103</b>
10x Combination Buffer, 3 x 1.5 ml	<b>A303103</b>
5x PCR Buffer RED, 6 x 1,5 ml **	<b>A301810</b>
PCR Grade Water, 6 x 5 ml	<b>A360056</b>

\*Ammonium Buffer, Standard Buffer and Combination Buffer are also available as Mg<sup>2+</sup> free buffers, detergent free buffers and Mg<sup>2+</sup> and detergent free buffers. \*\*For direct gel loading and visualisation.

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Other product sizes, combinations and customized solutions are available. Please look at [www.ampliqon.com](http://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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