

## TEMPase Hot Start Master Mix

with TEMPase Buffer II

### 2.0 Master Mix Kit (1.5mM MgCl<sub>2</sub>)

Catalogue no.: 230703 (500 reactions)

Cat.no.	Size reactions	TEMPase Hot Start Master Mix	Final MgCl <sub>2</sub> Conc.
230301	100	TEMPase Hot Start with TEMPase Buffer I	1.5mM
230303	500	TEMPase Hot Start with TEMPase Buffer I	1.5mM
230304	1000	TEMPase Hot Start with TEMPase Buffer I	1.5mM
230306	2500	TEMPase Hot Start with TEMPase Buffer I	1.5mM
230701	100	TEMPase Hot Start with TEMPase Buffer II	1.5mM
230703	500	TEMPase Hot Start with TEMPase Buffer II	1.5mM
230704	1000	TEMPase Hot Start with TEMPase Buffer II	1.5mM
230706	2500	TEMPase Hot Start with TEMPase Buffer II	1.5mM

Store at -20°C. Reagent for in-vitro laboratory use only

#### General Description

TEMPase Hot Start Master Mix is a ready-to-use 2.0X master mix. Simply add primers, template, and water to successfully carry out primer extensions and other molecular biology applications.

Ampliqon TEMPase Hot Start DNA Polymerase, the balanced K/NH<sub>4</sub><sup>+</sup> buffer system, dNTPs and magnesium chloride are present in TEMPase Hot Start Master Mix with TEMPase Buffer II. Each reaction requires 25 µL of the 2.0X reaction mix. Simply add primers, template and water to a total reaction volume of 50 µL.

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.

TEMPase Hot Start Master Mix offers several advantages. Set up time is significantly reduced. The chance of contaminating component stocks is eliminated. Reduction of reagent handling steps leads to better reproducibility. Standard tests can be set up with the confidence that results will be consistent every time.

#### Composition of 2.0X TEMPase HotStart MasterMix II

- Tris-HCl, pH 8.7, Balanced KCl/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 15 mM MgCl<sub>2</sub>, 1% Tween 20®.
- 0.4 mM dNTPs
- 0.2 units/µL TEMPase Hot Start DNA Polymerase
- Stabilizer

#### Suggested Protocol using TEMPase Hot Start Master Mix

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 individually determined.

#### Notes:

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis.
- The table below shows the reaction set up for a final volume of 50 µL.
- **Important:** Mix the solutions completely before use to avoid localized concentrations of salts.

1. Set up each reaction as follows:

Component	Vol./Reaction	Final Conc.
TEMPase Hot Start Master Mix with TEMPase Buffer II	25 µL	1X
Primer A	Variable	0.1–1.0 µM
Primer B	Variable	0.1–1.0 µM
Distilled Water	Variable	----
Template DNA	Variable	Variable
<b>TOTAL volume</b>	50 µL	----

- Mix gently by pipetting the solution up and down a few times.
- 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.**

For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

- Place the tubes in the thermal cycler and start the reaction.

### Three-step PCR Programme

Cycles	Duration of cycle	Temperature
1	15 minutes <sup>a</sup>	95 °C
25-35	20 - 30 seconds <sup>b</sup>	95 °C
	20 – 40 seconds <sup>c</sup>	50-65 °C
	30 seconds <sup>d</sup>	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 DNA 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 degrees Celsius below the T<sub>m</sub> 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.

### Related Products

Description	Cat. No.
Taq DNA Polymerase (1000 units) Glycerol free	<b>100103</b>
Taq DNA Polymerase (500 units) with 10X Ammonium Reaction Buffer with 10X Standard Reaction Buffer	<b>110303</b>
Taq DNA Polymerase (500 units) with 10X Combination Buffer	<b>110403</b>
Taq DNA Polymerase (500 units) with 10X Mg <sup>++</sup> Free Ammonium Buffer	<b>110503</b>
Taq DNA Polymerase 2.0X Master Mix (100 r) with 2.0 mM MgCl <sub>2</sub>	<b>150301</b>
Taq DNA Polymerase 2,0X MaMi RED (100 r) with 1.5 mM MgCl <sub>2</sub> ,	<b>180301</b>
Taq DNA Polymerase 2.0X MaMi RED (100 r) with 2.0 mM MgCl <sub>2</sub>	<b>190301</b>
AccuPOL DNA Polymerase (500 units)	<b>210303</b>
TEMPase Hot Start DNA Polymerase (500 units) with 10X TEMPase Buffer I with 10X TEMPase Buffer II	<b>220303</b>
TEMPase Hot Start 2 x Master Mix with TEMPase Buffer I (100 r)	<b>230301</b>
TEMPase Hot Start 2 x Master Mix with TEMPase Buffer II (100 r)	<b>230701</b>
TEMPase Hot Start 2 x Master Mix Blue With TEMPase Buffer I (100 r)	<b>230301</b>
TEMPase Hot Start 2 x Master Mix Blue With TEMPase Buffer II (100 r)	<b>230701</b>
RealQ PCR 2 x Master Mix (200 reaction) For probe	<b>250407</b>
RealQ PCR 2 x Master Mix (200 reaction) With green dye	<b>250507</b>
dNTP Mix (2 x 500µl) (12.5 mM of each dA, dC, dG and dT)	<b>501004</b>
dNTP Mix, (2 x 500 µl) (10 mM of each dA, dC, dG and dT),	<b>502004</b>

(Other product sizes available)

Tween 20® is a registered trademark of ICI Americas, Inc.

#### NOTICE

In certain countries, patents cover the PCR process. This product is intended for researchers having a license to perform PCR or those not required to obtain a license.

Ampliqon A/S  
Literbuen 11  
DK-2740 skovlunde  
Denmark

Phone: +45 70201169  
Fax: +45 70201179

www.ampliqon.com  
info@ampliqon.com

Issued 09/2010