Ampliqon
Bioreagents & PCR Enzymes

TEMPase Hot Start DNA Polymerase

With 10 x TEMPase Buffer II ($MgCl_2$ 15mM)

Concentration: 5 units/µl

Catalogue no.: 232706 (2500 units)

Cat. no.	Size units	10 x TEMPase Buffer II ($MgCl_2$ 15mM)	$MgCl_2$ 25 mM
232702	250	1.5 ml	1.5 ml
232703	500	1.5 ml	1.5 ml
232704	1000	2 x 1.5 ml	2x 1.5 ml
232706	2500	4 x 1.5 ml	4x 1.5 ml

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

General Description

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.
Once the reaction reaches optimal activating temp-erature, the chemical moiety is cleaved during a 15 minute heat activation step, releasing the active TEMPase Hot Start DNA Polymerase into the reaction.

Sensitivity improves multiplex PCR, an applied PCR technique that amplifies several specific targets simultaneously. Applications that previously required two or more reactions can be performed in a single reaction tube. Hence, multiplexing represents a substantial savings of time and costly reagents.

10x TEMPase Buffer II
This is a new optimized buffer system with a balanced Ammonium/Potassium concentration. This buffer improves more complicated PCR systems such as multiplex PCR.

Key Features

- Automated TEMPase Hot Start enzyme for increased specificity and product yield
- Successful multiplex reactions saves time and reagents
- Designed to diminish the formation of non-specific product
- Detection of low target copy number

10X TEMPase Hot Start Buffer II
Tris-HCl, pH 8.7, Balanced $KCl/(NH_4)_2SO_4$, 15 mM $MgCl_2$, 1% Tween 20®.

TEMPase Hot Start Storage Buffer
Enzyme is supplied in 20 mM Tris-HCl pH 8.3, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, 0.5% NP40, 50% glycerol.

Unit Definition
One unit is defined as the amount that incorporates 10 nmoles of dNTPs into acid-precipitable form in 30 minutes at 72°C under standard assay conditions.

Quality Control
Endonuclease, exonuclease and priming activities are not detected after 3 hours incubation of 1 µg of pUC19 plasmid DNA and 0.5 µg EcoR I digested lambda phage DNA at 72°C in the presence of 40 units of TEMPase DNA Polymerase.

Suggested Protocol using TEMPase Hot Start DNA Polymerase
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.
- 15 mM $MgCl_2$ is present in the 10X TEMPase Buffer II. The 1X concentration is 1.5mM $MgCl_2$.
- In some applications, more than 1.5mM $MgCl_2$ is needed for the best results. For this reason, 25mM $MgCl_2$ is included with the kit. Table 2 provides the volume of 25mM $MgCl_2$ to add to the master mix if a higher $MgCl_2$ concentration is required.

1. Thaw 10X TEMPase Buffer II, dNTP mix, primer solutions. **It is important to mix the solutions completely before use to avoid localized concentrations of salts**.

2. Prepare a master mix according to Table 1. The master mix typically contains all the components needed for extension except the template DNA.

Table 1. Reaction components (master mix and template DNA)

Component	**Vol./reaction**	**Final Conc.**
10X TEMPase Buffer II	5 µL	1X
dNTP mix (12.5 mM of each)	0.8 µL	0.2 mM of each dNTP
Primer A	Variable	0.1–1.0 µM
Primer B	Variable	0.1–1.0 µM
TEMPase Hot Start DNA Polymerase	1 µL	5 units
Distilled Water	Variable	- - - -
Template DNA	Variable	Variable
TOTAL volume	50 µL	- - - -

Table 2. $MgCl_2$ concentration in a 50 µL reaction

Final $MgCl_2$ conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25 mM $MgCl_2$ per reaction (µL):	0	1	2	3	4	5	6

3. Mix the master mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, e.g., by pipetting the master mix up and down a few times.

4. Add template DNA to the individual tubes containing the master 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.**

   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.

Three-step PCR Programme

Cycles	Duration of cycle	Temperature
1	15 minutes[a]	95 °C
25-35	20 - 30 seconds[b]	95 °C
	20 – 40 seconds[c]	50-65 °C
	30 seconds[d]	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 DNA 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 degrees Celsius below the Tm 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.

Related Products

Description	Cat. No.
Taq DNA Polymerase (1000 units) Glycerol free	**100103**
Taq DNA Polymerase (500 units) with 10X Ammonium Reaction Buffer with 10X Standard Reaction Buffer	**110303**
Taq DNA Polymerase (500 units) with 10X Combination Buffer	**110403**
Taq DNA Polymerase (500 units) with 10X Mg^{++} Free Ammonium Buffer	**110503**
Taq DNA Polymerase 2.0X Master Mix (100 r) with 2.0 mM MgCl2	**150301**
Taq DNA Polymerase 2,0X MaMi RED (100 r) with 1.5 mM MgCl2,	**180301**
Taq DNA Polymerase 2.0X MaMi RED (100 r) with 2.0 mM MgCl2	**190301**
AccuPOL DNA Polymerase (500 units)	**210303**
TEMPase Hot Start DNA Polymerase (500 units) with 10X TEMPase Buffer I with 10X TEMPase Buffer II	**220303**
TEMPase Hot Start 2 x Master Mix with TEMPase Buffer I (100 r)	**230301**
TEMPase Hot Start 2 x Master Mix with TEMPase Buffer II (100 r)	**230701**
TEMPase Hot Start 2 x Master Mix Blue With TEMPase Buffer I (100 r)	**230301**
TEMPase Hot Start 2 x Master Mix Blue With TEMPase Buffer II (100 r)	**230701**
RealQ PCR 2 x Master Mix (200 reaction) For probe	**250407**
RealQ PCR 2 x Master Mix (200 reaction) With green dye	**250507**
dNTP Mix (2 x 500µl) (12.5 mM of each dA, dC, dG and dT)	**501004**
dNTP Mix, (2 x 500 µl) (10 mM of each dA, dC, dG and dT),	**502004**

(Other product sizes available)

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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.

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