Ampliqon

TEMPase Hot Start DNA Polymerase

With 10XTEMPase Buffer I ($MgCl_2$ 15mM)
With 10XTEMPase Buffer II ($MgCl_2$ 15mM)

Conc.: 5 units/µl

Cat. No.: 220304 (1.000 Units)

Cat. No.	Size Units	10X TEMPase Buffer I ($MgCl_2$ 15mM)	10X TEMPase Buffer II ($MgCl_2$ 15mM)	$MgCl_2$ 25 mM
220302	250	1.5 mL	1.5 mL	1.5 mL
220303	500	1.5 mL	1.5 mL	1.5 mL
220304	1000	2 x 1.5 mL	2 x 1.5 mL	2x 1.5 mL
220306	2500	4 x 1.5 mL	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 temperature, 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.

included in the kit; 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 I

Tris-HCl, pH 8.5 $(NH_4)_2S0_4$, 15 mM $MgCl_2$, 1% Tween 20®.

10X TEMPase Hot Start Buffer II

Tris-HCl pH 8.7, Balanced KCl/$(NH_4)_2S0_4$, 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 *Eco*R 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 I and 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 I or/and 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 I or 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.

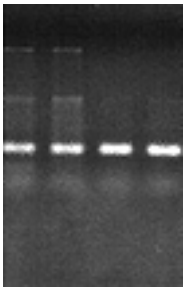

Figure 1. Comparison of Taq DNA polymerase with Ampliqon TEMPase Hot Start DNA polymerase.
Under standard amplification conditions, a 355 bp DNA fragment was amplified using either a standard Taq DNA polymerase or TEMPase Hot Start DNA polymerase.

Lane 1 5 units of Taq DNA Polymerase
Lane 2 2.5 units of Taq DNA Polymerase
Lane 3 5 units of TEMPase Hot Start, with 15 minutes activation step at 95°C before cycling
Lane 4 2.5 units of TEMPase Hot Start, with 15 minutes activation step at 95°C before cycling

Related Products

Description	Cat. No.
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 Reac) *with 2.0 mM MgCl2*	**150301**
Taq DNA Polymerase 2,0X MaMi RED (100 Reac) *with 1.5 mM MgCl2,*	**180301**
Taq DNA Polymerase 2.0X MaMi RED (100 Reac) *with 2.0 mM MgCl2*	**190301**
AccuPOL DNA Polymerase (500 Units)	**210303**
TEMPase Hot Start DNA Polymerase (500Units) *with 10X TEMPase Buffer I* *with 10X TEMPase Buffer II*	**220303**
UniPOL –Long Range PCR (100 Reac)	**270701**
Rapid Ligation Kit (50 React)	**750300**
RT-PCR One Tube (100 Reac)	**740301**
TEMPase Hot Start 2X Master Mix *with TEMPase Buffer I* (100 Reac)	**230301**
TEMPase Hot Start 2X Master Mix *with TEMPase Buffer II* (100 Reac)	**230701**
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**
GC5 Value Efficiency, 10^8 Cfu/µg pUC19 Chemically Competent Cells, (10x 200µl)	**812010**
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells, (10x 50µl)	**805010**
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells, (5x 200µl)	**802005**
SuperPath GC10, 10^{10} Cfu/µg pUC19 ElectroCompetent Cells, (5x 80µl)	**830805**
SOC Medium, 10x 10mL	**800000**

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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.