AMPLIQON
PCR ENZYMES & REAGENTS

15 MIN HOT START

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TEMPase Hot Start DNA Polymerase Glycerol Free

Concentration: 5 units/µl

MADE IN DENMARK

Cat. No.	Units	TEMPase Hot Start DNA Polymerase Glycerol Free ID: 5101700
A240003	500	100 µl
A240004	1000	2 x 100 µl
A240006	2500	5 x 100 µl
A240007	5000	10 x 100 µl
A240010	25,000	1 x 5 ml

Key Features

TEMPase Hot Start DNA Polymerase, Glycerol Free, is a high-quality DNA polymerase developed for automation. It is a glycerol free formulation of TEMPase Hot Start Polymerase with the same excellent performance. The glycerol free formulation makes it well suited for automation and freeze-drying.

TEMPase Hot Start DNA Polymerase is a modified form of Ampliqon Taq DNA Polymerase. 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. 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.

Kit Components

TEMPase Hot Start DNA Polymerase in Glycerol Free Storage Buffer

5 U/µl TEMPase Hot Start DNA Polymerase, 20 mM Tris-HCl pH 8.9, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween® 20.

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 1 month.

Quality Control

TEMPase Hot Start DNA Polymerase is tested for contaminating activities, with no traces of endonuclease activity, nicking activity or exonuclease activity.

Unit Definition

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

Protocol

This protocol serves as a guideline to ensure optimal PCR results when using TEMPase Hot Start DNA Polymerase, Glycerol free. Optimal reaction conditions such as incubation times, temperatures and amount of template DNA may vary and must be determined individually.

1. Thaw Solutions. **It is important to thaw all solutions completely (some buffers need to reach room temperature) and mix thoroughly before use to avoid localized concentrations of salts**. Keep all components on ice.

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 mix and template DNA

Component	Vol./reaction*	Final concentration*
10x Buffer	5 µl	1x
25 mM $MgCl_2$	3 µl (2 – 9 µl)	1.5 mM (1 – 4.5 mM)
dNTP mix (10 mM each)	1 µl	0.2 mM of each dNTP
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)
TEMPase DNA Pol.	0.4 µl (0.2 – 1 µl)	2 units (1 – 5 units)
PCR-grade H_2O	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)
TOTAL volume	50 µl	-

* Suggested starting conditions; theoretically used conditions in brackets. The final volume can be reduced to 25 µl by using half of the volumes suggested in Vol./reaction, e.g. 0.2 µl TEMPase instead of 0.4 µl TEMPase.

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.**
   For maximum yield and specificity, temperatures and cycling times should be optimized for each new template 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[a]	95 °C
25 – 35	20 – 30 seconds[b] 20 – 40 seconds[c] 30 – 90 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.

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.

Notes:

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

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Other product sizes, combinations and customized solutions are available. Please look at 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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