AMPLIQON
PCR ENZYMES & REAGENTS

15 MIN HOT START

Advion Interchim scientific

GC-Rich DNA Target Kit

With 4x GC Buffer I (6 mM $MgCl_2$)
With 4x GC Buffer II (6 mM $MgCl_2$)

Concentration: 5 units/µl TEMPase Hot Start Polymerase

MADE IN DENMARK

Cat. No.: A227103
500 Units

A227103

-	TEMPase Polymerase 5 U/µl	4x GC Buffer I	4x GC Buffer II	$MgCl_2$ 25 mM
		6 mM $MgCl_2$		
ID No.	**5101650**	**5100710**	**5100720**	**5575801**
Cap colour	**Red**	**Purple**	**Black**	**Clear**
Content	100 µl	1.5 ml	1.5 ml	1.5 ml

Key Features

Ampliqon offers a product series specifically developed for the amplification of GC-rich DNA sequences. The Ampliqon TEMPase Hot Start DNA Polymerase combined with GC Buffer I or GC Buffer II promote excellent amplification results with targets of varying degrees of GC content.

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 minutes heat activation step, releasing the active TEMPase Hot Start DNA Polymerase into the reaction.

The GC Buffers in combination with Ampliqon TEMPase Hot Start DNA Polymerase and the heat activation step result in a high success rate in amplification of DNA targets with high GC content.

Kit Components

TEMPase Hot Start DNA Polymerase in 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, 50% glycerol.

4x GC Buffer I

Optimized buffer components, 6 mM $MgCl_2$.

4x GC Buffer II

Optimized buffer components, 6 mM $MgCl_2$.

$MgCl_2$

25 mM $MgCl_2$ in PCR grade water.

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

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 GC-RICH DNA Target Kit. Optimal reaction conditions such as incubation times, temperatures and amount of template DNA may vary and must be determined individually.

1. Thaw 4x Buffer, dNTP mix and primer solutions. **It is important to thaw the solutions completely (some buffers need to reach room temperature) and mix thoroughly before use to avoid localized concentrations of salts**. The polymerase is provided in glycerol and does not need thawing.

   Important: Spin vials briefly before use.

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

Table 1. Reaction mix and template DNA

Component	Vol./reaction*	Final concentration*
4x Buffer	12.5 µl	1x
25 mM $MgCl_2$	0 µl (0 – 6 µl)	1.5 mM (1.5 – 4.5 mM)
dNTP mix (10 mM each)	1.25 µl	0.25 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, eg. 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	30 seconds[b] 40 – 60 seconds[c] 40 – 60 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:

- 6 mM $MgCl_2$ is present in the Ampliqon 4x buffers. The 1x concentration is 1.5 mM $MgCl_2$. In some applications, more than 1.5 mM $MgCl_2$ is required for best results. For this reason, 25 mM $MgCl_2$ is included in the kit. Table 3 provides the volume of 25 mM $MgCl_2$ to be added to the reaction mix if a higher $MgCl_2$ concentration is required.

Table 3. Additional volume (µl) of $MgCl_2$ per 50 µl reaction

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

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

Related Products

TEMPase DNA Polymerase (500 units) *	Cat. No.
TEMPase Hot Start DNA Polymerase 5 U/µl	**A220003**
• with 10x Ammonium Buffer	**A221103**
• with 5x PCR Buffer RED	**A221803**
TEMPase Hot Start DNA Polymerase 5 U/µl, glycerol free	**A240003**
• with 10x Ammonium Buffer	**A241103**

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

*Available in kits including one or two buffers (Ammonium Buffer, Standard Buffer or Combination Buffer). All kits include extra 25 mM $MgCl_2$.

Buffers for DNA polymerases *	Cat. No.
10x Ammonium Buffer, 3 x 1.5 ml	**A301103**
10x Standard Buffer, 3 x 1.5 ml	**A302103**
10x Combination Buffer, 3 x 1.5 ml	**A303103**
5x PCR Buffer RED, 6 x 1,5 ml **	**A301810**

*Ammonium Buffer, Standard Buffer and Combination Buffer are also available as Mg^{2+} free buffers, detergent free buffers and Mg^{2+} and detergent free buffers.
**For direct gel loading and visualisation.

TEMPase Hot Start Master Mixes (500 x 50 µl reactions) *	Cat. No.
2x Master Mix A**, 1.5 mM $MgCl_2$ final concentration	**A230303**
2x Master Mix A**BLUE, 1.5 mM $MgCl_2$ final concentration	**A290403**

*Master mixes available also in 1.1x variants as well as 2 mM $MgCl_2$ variants, **Mix A is Ammonium Buffer based, also available as Mix C based on Combination Buffer.

Special Master Mixes (500 x 50 µl reactions)	Cat. No.
Multiplex 2x Master Mix, 3 mM $MgCl_2$ final concentration	**A260303**
GC TEMPase 2x Master Mix I – for GC-rich templates	**A331703**
GC TEMPase 2x Master Mix II – for GC-rich templates	**A332703**

Ultrapure dNTPs*	Cat. No.
dNTP Mix 40 mM (2 x 500 µl): 10 mM each dA, dC, dG, dT	**A502004**
dNTP Set, 100 mM each: 250 µl of each dA, dC, dG and dT	**A511104**

*Other concentrations and Single dNTPs are available.

Loading Buffers, PCR water and Ladders	Cat. No.
5x Loading Buffer Red *, 5 x 1 ml	**A608104**
Iqon PCR Ladder **, 100 – 3000 bp, 1 x 0.5 ml	**A610341**
PCR Grade Water, 6 x 5 ml	**A360056**

* Also available with Blue, Orange or Cyan. ** Available in different size ranges.

For Research Use Only. Not for use in diagnostics procedures.

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

Issued 08/2021

Advion Interchim scientific

Ampliqon A/S, Stenhuggervej 22, DK-5230 Odense M, Denmark. Phone: +45 70201169 Fax: +45 70201179 info@ampliqon.com www.ampliqon.com