AMPLIQON
PCR ENZYMES & REAGENTS

15 MIN HOT START

DryTech TEMPase 5x Master Mix Green

2 mM $MgCl_2$ final concentration

Cat. No.: A747306
2500 reactions
Reaction size 25 µl

A747306

MADE IN **DENMARK**

	Foil bags 5300610-0500 with:	
Content	**5 x 2 Vials**	**10 x 1.3 ml**
	DryTech TEMPase 5x Master Mix 2 mM $MgCl_2$ final conc.	**5x DryTech Buffer Green**
ID No.	**5300600-0250**	**5100410-1300**
Cap colour	**Blue**	**Black**

Key Features

- Lyophilized TEMPase Hot Start DNA polymerase Master Mix
- Green dye for direct loading
- Highly stable at room temperature
- Shipping at ambient temperature
- Storage at room temperature
- Reduced CO_2 impact
- For amplification up to 4 kb

DryTech TEMPase 5x Master Mix Green is lyophilized for storage at room temperature and shipping at ambient temperature. The lyophilized DryTech TEMPase 5x Master Mix is easily reconstituted with the 5x DryTech Buffer Green included in the kit.

The reconstituted DryTech TEMPase 5x Master Mix Green is a ready-to-use 5x reaction mix with everything needed to perform PCR; Ampliqon TEMPase Hot Start DNA polymerase, optimized NH_4^+ PCR buffer system, dNTPs and magnesium chloride present. Each reaction requires 5 µl of the 5x Master Mix. Simply add primers, template and water to a total reaction volume of 25 µl to successfully carry out PCR.

There is no need to buy and use separate loading dyes. Simply load a portion of the reaction product onto an agarose gel for electrophoresis and subsequent visualization. The green dye separates into a yellow and blue tracking front during electrophoresis.

DryTech TEMPase 5x Master Mix Green is a cost saving choice as it can be shipped at ambient temperature without the presence of dry ice. Furthermore, the reduced electricity consumption and greenhouse gas emission leave an improved CO_2 imprint.

Recommended Shipping, Storage and Stability

- Shipping at ambient temperature.
- The lyophilized 5x master mix and the 5x DryTech Buffers are stable at room temperature (≤ 25 °C) for 12 months from date of receipt.
- The reconstituted master mix is stable for 6 months at -20 °C or for 3 months at +4 °C.

Quality Control

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

Protocol

This protocol serves as a guideline to ensure optimal PCR results when using DryTech TEMPase 5x Master Mix Green. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

DryTech TEMPase 5x Master Mix has to be reconstituted using the 5x DryTech Green Buffer provided in the kit.

1. Reconstitute each vial of DryTech TEMPase 5x Master Mix with 1.25 ml of 5x DryTech Buffer Green. **Tip:** Add 2 x 625 µl using reverse pipetting. Vortex for 30 seconds then incubate at RT for 1 min and finally vortex again until fully dissolved.

Important: After reconstitution the DryTech TEMPase 5x Master Mix Clear should be stored at -20 °C or +4 °C.

2. Thaw the reconstituted DryTech TEMPase 5x Master Mix Green and primers. **It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts**.

3. Prepare a reaction mix. Table 1 shows the reaction set up for a final volume of 25 µL. If desired, the reaction size may be scaled down. Use 5 µl of the DryTech TEMPase 5x Master Mix in a final volume of 25 µl.

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

Component	Vol./reaction*	Final concentration*
DryTech 5x MM	5 µl	1x
25 mM $MgCl_2$	0 µl (0 – 2 µl)	2 mM (2 – 4 mM)
Primer A (10 µM)	0.5 µl (0.25 – 2.5 µl)	0.2 µM (0.1 – 1.0 µM)
Primer B (10 µM)	0.5 µl (0.25 – 2.5 µl)	0.2 µM (0.1 – 1.0 µM)
PCR-grade H_2O	X µl	-
Template DNA	X µl	Genomic DNA: 50 ng (10 – 500 ng) Plasmid DNA: 0.5 ng (0.1 – 1 ng) Bacterial DNA: 5 ng (1 – 10 ng)
TOTAL volume	25 µl	-

* Suggested starting conditions; theoretically used conditions in brackets

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

5. Add template DNA to the individual tubes containing the reaction mix.

6. Program the thermal cycler according to the manufacturer´s instructions. See table 2 for an example.
   For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

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

8. At the end of the run, simply load a portion of the reaction product (e.g. 10 µl) onto an agarose gel for analysis.

Table 2. Three-step PCR program

Cycles	Duration of cycle	Temperature
1	15 minutes	95 °C
25 - 35	20 – 30 seconds[a] 20 – 40 seconds[b] 30 seconds[c]	95 °C 50 – 65 °C 72 °C
1	5 minutes[d]	72 °C

[a.] 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.

[b.] 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.

[c.] 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.

[d.] 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:

- The final $MgCl_2$ concentration of this 5x Master Mix is 2 mM. In some applications, more than 2 mM $MgCl_2$ is required for best results. Use 25 mM to adjust the Mg^{2+} concentration according to table 3.

Table 3. Additional volume (µl) of $MgCl_2$ per 25 µl reaction:

Final $MgCl_2$ conc. in reaction (mM)	2	2.5	3.0	3.5	4.0	4.5
Volume of 25 mM $MgCl_2$	0	0.5	1	1.5	2	2.5

Related Products

DryTech TEMPase 5x Master Mix Clear	Cat. No.
500 reactions	**A747203**
1000 reactions	**A747204**
2500 reactions	**A747206**

DryTech TEMPase 5x Master Mix Green	Cat. No.
500 reactions	**A747303**
1000 reactions	**A747304**
2500 reactions	**A747306**

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

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