AMPLIQON
PCR ENZYMES & REAGENTS

15 MIN HOT START

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DryTech TEMPase 5x Master Mix Green

2 mM $MgCl_2$ final concentration

MADE IN DENMARK

Cat. No.	Reactions (25 µl)	DryTech TEMPase 5x Master Mix ID: 5300610	5x DryTech Buffer Green ID: 5100410
A747303	500	1 x 4 tubes*	2 x 1.3 ml
A747304	1000	2 x 4 tubes*	4 x 1.3 ml
A747306	2500	5 x 4 tubes*	10 x 1.3 ml
A747399	Sample – 50	1 tube**	1 x 1.3 ml

*Packed in foil bags with 4 tubes in each (ID: 5300610-0500).
**Packed in a foil bag with 1 tube (ID: 5300610-0050).

Introduction

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. 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.

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

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 must be reconstituted using the 5x DryTech Green Buffer provided in the kit.

1. Reconstitute each vial of DryTech TEMPase 5x Master Mix with 625 µl of 5x DryTech Buffer Green. 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 Green 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.

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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)	2.0	2.5	3.0	3.5	4.0	4.5
Volume of 25 mM $MgCl_2$	0	0.5	1.0	1.5	2.0	2.5

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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