AMPLIQON
PCR ENZYMES & REAGENTS

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Taq DNA Polymerase 2x Master Mix RED

MADE IN DENMARK

Cat. No.	Reactions (50 µl)	Taq DNA Polymerase 2x Master Mix RED, 1.5 mM $MgCl_2$ ID: 5200300
A180301	100	2 x 1.25 ml
A180303	500	10 x 1.25 ml
A180306	2500	50 x 1.25 ml
A180307	5000	25 x 5 ml
A180308	10,000	28 x 9 ml
A180399	Sample - 20	0.5 ml

Key Features

Taq DNA Polymerase 2x Master Mix RED is a ready-to-use 2x reaction mix with the Ampliqon Taq DNA polymerase, the NH_4^+ buffer system, dNTPs and magnesium chloride present. Each reaction requires 25 µl of the 2x Master Mix RED. Simply add primers, template and water to a total reaction volume of 50 µl.

Taq DNA Polymerase 2x Master Mix RED offers several advantages. Setup time is significantly reduced. The risk of contaminating component stocks is eliminated. Reduction of reagent handling steps leads to better reproducibility. Standard tests can be set up with the confidence that results will be consistent every time.
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 red dye front runs at 300 – 1000 bp on a 0.5 – 1.5% agarose gel.

Composition of the Taq DNA Polymerase 2x Master Mix RED (1.5 mM $MgCl_2$ final concentration)

- Tris-HCl pH 8.5, $(NH_4)_2S0_4$, 4 mM $MgCl_2$, 0.2% Tween® 20
- 0.4 mM of each dNTP
- Ampliqon Taq DNA polymerase
- Inert red dye and stabilizer

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

Taq 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 Taq DNA Polymerase 2x Master Mix RED. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

1. Thaw Taq 2x Master Mix RED and primers. **It is important to thaw the solutions completely and mix thoroughly before use to avoid localized concentrations of salts**. Keep all components on ice.

2. Prepare a reaction mix. Table 1 shows the reaction setup for a final volume of 50 µL. If desired, the reaction size may be scaled down. Use 10 µl of the Taq 2x Master Mix RED for a final volume of 20 µl.

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

Component	Vol./reaction*	Final concentration*
Taq 2x Master Mix	25 µl	1x
25 mM $MgCl_2$	0 µl (0-6 µl)	1.5 mM (1.5-4.5 mM)
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)
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	50 µl	-

* Suggested starting conditions; theoretically used conditions in brackets

3. 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.

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

5. 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.

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

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

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Table 2. Three-step PCR program

Cycles	Duration of cycle	Temperature
1	2-5 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: Taq 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.

Two-step PCR program

Fast 2-step PCR protocols are available using this link:
https://ampliqon.com/en/pcr-technology/application-notes/

Notes:

- The final $MgCl_2$ concentration of this 2x Taq Master Mix RED is 1.5 mM. In some applications, more than 1.5 mM $MgCl_2$ is required for best results. Use 25 mM $MgCl_2$ to adjust the Mg^{2+} concentration according to table 3.

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

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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

Issued 06/2025

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