AMPLIQON
PCR ENZYMES & REAGENTS

Taq DNA Polymerase 2x Master Mix

1.5 mM $MgCl_2$ final concentration

Cat. No.: A140306

A140306

Cat. No.	Size Reactions	Taq DNA Polymerase 2x Master Mix, 1.5 mM $MgCl_2$
ID No. Cap colour	-	5200100 Red
A140306	2500	50 x 1.25 ml

Key Features

- High performance thermostable DNA polymerase
- Significantly reduced set up time
- Increased reproducibility
- Diminished risk of contamination
- No proofreading – lacks a 3'→5' exonuclease activity
- Ideal for TA cloning – leaves an A' overhang

Taq DNA Polymerase 2x Master Mix 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. Simply add primers, template and water to a total reaction volume of 50 µl to successfully carry out primer extensions and other molecular biology applications.

Taq DNA Polymerase 2x Master Mix offers several advantages. Set up time is significantly reduced. The chance 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.

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

- Tris-HCl pH 8.5, $(NH_4)_2S0_4$, 3 mM $MgCl_2$, 0.2% Tween® 20
- 0.4 mM of each dNTP
- 0.2 units/µl Ampliqon Taq DNA polymerase
- 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.

Unit Definition

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

Protocol

This protocol serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

Notes:

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis. Work on ice at all times.
- The final $MgCl_2$ concentration of this 2x Taq Master Mix is 1.5 mM. In some applications, more than 1.5 mM $MgCl_2$ is required for best results. Use 25 mM $MgCl_2$ (see Additional Products) to adjust the Mg^{2+} concentration according to table 1.

Table 1. 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

1. Thaw Taq 2x Master Mix 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. Set up each reaction. Table 2 shows the reaction set up 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 in a final volume of 20 µl.

Table 2. Reaction components (reaction mix and template DNA)

Component	Vol./reaction*	Final concentration*
Taq 2x Master Mix	25 µl	1x
25 mM $MgCl_2$	X µl	1.5 mM (0.5 – 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 3 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.

Table 3. 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.

Related Products

Taq Polymerase (500 units) *	Cat. No.
Taq DNA Polymerase 5 U/µl	**A110003**
• with 10x Ammonium Buffer	**A111103**
• 5x PCR Buffer RED	**A111803**
Taq DNA Polymerase 5 U/µl, RED	**A200003**
• with 10x Ammonium Buffer	**A201103**
Taq DNA Polymerase 5 U/µl, glycerol free	**A100003**
• with 10x Ammonium Buffer	**A101103**

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

High Fidelity - Proof reading (500 units) **	Cat. No.
AccuPOL DNA Polymerase 2.5 U/µl	**A210003**
• with 10x Ammonium Buffer	**A211103**

*Available in kits including one or two buffers (Ammonium Buffer, Standard Buffer or Combination Buffer). **AccuPOL only available in kits with Ammonium 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.

Taq Master Mixes (500 x 50 µl reactions) *	Cat. No.
2x Master Mix, 1.5 mM $MgCl_2$ final concentration	**A140303**
2x Master Mix RED, 1.5 mM $MgCl_2$ final concentration	**A180303**

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**

Real-time PCR Master Mixes (400 x 25 µl reactions)	Cat. No.
RealQ Plus 2x Master Mix for probe,	
• without ROX™	**A313402**
• with low ROX™	**A314402**
• with high ROX™	**A315402**
RealQ Plus 2x Master Mix Green	
• without ROX™	**A323402**
• with low ROX™	**A324402**
• with high ROX™	**A325402**

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 and Ladders	Cat. No.
5x Loading Buffer Red *, 5 x 1 ml	**A608104**
PCR DNA Ladder **, 100 – 3000 bp, 1 x 0.5 ml	**A610341**

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

Reagents for *in vitro* laboratory use only.

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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