

AQ97 High Fidelity DNA Polymerase 2x Master Mix

MADE IN DENMARK



A770106

Cat. No.: A770106
2500 Reactions

AQ97 High Fidelity DNA Polymerase 2x Master Mix 3 mM MgCl ₂	
ID No.	5500450
Cap colour	Blue
Content	50 x 1.25 ml

Key Features

- Convenient reaction set-up
- High fidelity: > 60x Taq¹⁾
- Long range amplification: 11 kb for gDNA
- High elongation rate: 10 sec/kb
- Excellent performance on a vast range of amplicons (high AT and high GC)
- Recommended for cloning, mutagenesis and other molecular applications requiring extremely high fidelity

AQ97 High Fidelity DNA Polymerase 2x Master Mix is an all-in-one 2x master mix containing the AQ97 High Fidelity DNA Polymerase, AQ97 Buffer, dNTPs and MgCl₂. Simply mix AQ97 High Fidelity DNA Polymerase 2x Master Mix with primers, DNA template and water and you are ready to carry out PCR.

AQ97 High Fidelity DNA Polymerase is a thermostable, chimeric DNA Polymerase created specifically for low-bias, high fidelity amplification of a vast range of amplicons. AQ97 High Fidelity DNA Polymerase delivers high-speed elongation and processivity, due to its fusion with a DNA-binding domain.

¹⁾ Determined through a novel NGS-based analysis of nucleotide misincorporation during PCR

Protocol

Optimal reaction conditions such as incubation times, temperatures and amount of template DNA may vary and must be determined individually. Amplification of templates with high GC content, high secondary structures as well as long range amplification may require more optimization – for tips see section *Strategies for Optimization*

Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis. **Work on ice at all times.**

1. Thaw AQ97 High Fidelity DNA Polymerase 2x Master Mix and primer solutions.
It is recommended to completely thaw and thoroughly mix the master mix to ensure proper resuspension of precipitates.
2. Prepare the reaction mix. Table 1 shows the reaction set up for a final volume of 50 µl. If desired, the reaction size may be scaled down.

3. Mix the reaction mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, e.g. by pipetting the master 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. 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 1. Reaction components

Component	Vol./reaction*	Final concentration*
2x Master Mix	25 µl	1x
Primer A (10 µM)	1 µl	0.2 µM
Primer B (10 µM)	1 µl	0.2 µM
25 mM MgCl ₂	0 µl (0 – 6 µl)	1.5 mM (1.5 – 4.5 mM)
Betaine (5M)**	10 - 20 µl	1 – 2M
PCR-grade H ₂ O	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.

** Suggested for GC-rich amplification and long-range amplification. See section *Strategies for Optimization*.

Table 2. Three-step PCR program

Cycles	Duration of cycle	Temperature
1	2 min ^{a)}	98 °C
25 - 35	10 – 20 sec ^{a)} 15 – 30 sec ^{b)} 10 – 60 sec ^{c)}	98 °C 55 – 70 °C 72 °C
1	5 minutes	72 °C

^{a)} Denaturation: 2 min initial denaturation is sufficient for most templates. During thermocycling, 10 seconds usually works very well. Longer denaturation times might be required for long range PCR or amplification from templates with a high GC content.

^{b)} Primer annealing: Typically, the annealing temperature is about 3 – 5 °C below the T_m (melting temperature) of the primers used. **Because of the high salt content within the AQ97 High Fidelity DNA Polymerase 2x Master Mix, annealing temperature will likely be higher than with more traditional PCR master mixes.**

^{c)} Extension: The recommended extension temperature is 72°C. Extension times highly depends on the length of the amplicon. **Generally, we recommend an extension time of 10-30 seconds per kb for complex genomic targets. 10 seconds per kb is often sufficient for simpler targets (such as plasmid) or short complex targets (< 3 kb). 30-60 seconds per kb is recommended for long amplicons (> 3 kb).**

Strategies for Optimization:

Long-range amplification

- Longer extension times often resolve low-yield amplification of long amplicons.
- The addition of 1-2 M Betaine solution often improves reaction performance (See Additional Products for ordering information).
- Increased template concentration will increase product yield.
- Increased primer concentration can increase product yield for some reactions.

GC-rich amplification

- Addition of 1-2 M Betaine solution often improves reaction performance. (See Additional Products for ordering information)

Primers

- Primers of 20 – 40 nucleotides with a GC content of 40 - 60 % are recommended. Online Software such as the Primer3plus

<https://primer3plus.com/cgi-bin/dev/primer3plus.cgi> can be used to design primers.

MgCl₂

- The optimal MgCl₂ concentration should be determined empirically, but in most cases a final concentration of 1.5 mM, as provided in AQ97 High Fidelity DNA Polymerase 2x Master Mix, will produce satisfactory results. Table 3 provides the volume of 25 mM MgCl₂ to be added to the master mix if a higher MgCl₂ concentration is required.

Table 3. Additional volume (µl) of MgCl₂ 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

Kit Components

AQ97 High Fidelity DNA Polymerase 2x Master Mix

- AQ97 High Fidelity DNA Polymerase
- Optimized buffer components, 3.0 mM MgCl₂
- dNTPs

5 M Betaine Enhancer Solution

Sold separately. Cat No.: A351104

More info

Recommended Storage and Stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.

Optional: Store at +4 °C for up to 6 months.

Quality Control

AQ97 High Fidelity DNA Polymerase is tested for contaminating activities with no traces of endonuclease activity or nicking activity. Furthermore, long range capacity is tested on human gDNA target of 6 kb.

Related Products

AQ97 High Fidelity DNA Polymerase 2x Master Mix	Cat. No.
100 reactions	A770101
500 reactions	A770103
2500 reactions	A770106
5000 reactions	A470107

AQ97 High Fidelity DNA Polymerase 2 U/µl	Cat. No.
With 5x AQ97 Buffer – 100 U	A767501
With 5x AQ97 Buffer – 500 U	A767503
With 5x AQ97 Buffer – 1000 U	A767504
With 5x AQ97 Buffer – 2500 U	A767506

PCR Grade Water	Cat. No.
6 x 5 ml	A360056

Betaine Enhancer Solution 5 M	Cat. No.
5 x 1 ml	A351104

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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