

## AQ97 HiFi 2x Master Mix

1.5 mM MgCl<sub>2</sub> final concentration

Cat. No.: A770201



100 Reactions

MADE IN DENMARK

AQ97 HiFi 2x Master Mix	
ID No.	5500470
Cap colour	Blue
Content	2 x 1.25 ml

### Contents

All-in-one 2x master mix containing the AQ97 High Fidelity DNA Polymerase, optimized Buffer, dNTPs and MgCl<sub>2</sub>. Recommended for low-bias, high fidelity amplification.

### Recommended Storage and Stability

Temperature	Duration
Room temperature	Up to 5 days
4° C	Up to 6 months
-20° C	Long term. See expiry on tube

### Protocol

1. Thaw AQ97 HiFi 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. Combine master mix, primers, template DNA and water according to the following table.

Table 1: Recommended reaction setup

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 <sub>2</sub>	0 µl (0 – 6 µl)	1.5 mM (1.5 – 4.5 mM)
Betaine (5 M)**	10 - 20 µl	1 – 2 M
PCR-grade H <sub>2</sub> 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)
<b>TOTAL volume</b>	50 µl	-

\* Suggested starting conditions; theoretically used conditions in brackets.

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

3. Transfer the appropriate volume to a PCR plate or strip compatible with the chosen thermal cycler. Seal the plate/strip.

### 4. Three-step PCR program

Table 2: Recommended cycling conditions

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

<sup>a.</sup> Denaturation: 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.

<sup>b.</sup> Primer annealing: Typically, the annealing temperature is about 3 – 5 °C below the T<sub>m</sub> (melting temperature) of the primers used. **Because of the high salt content within the AQ97 HiFi 2x Master Mix, annealing temperature will likely be higher than with more traditional PCR master mixes.**

<sup>c.</sup> 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. For ordering information visit [ampliqon.com](http://ampliqon.com).
- 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.

##### Primers

- Primers of 20 – 40 nucleotides with a GC content of 40 - 60 % are recommended. Online Software such as the Primer3plus can be used to design primers.

##### MgCl<sub>2</sub>

- The optimal MgCl<sub>2</sub> concentration should be determined empirically, but in most cases a final concentration of 1.5 mM, as provided in AQ97 HiFi 2x Master Mix, will produce satisfactory results.

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](http://www.ampliqon.com) or ask for our complete product list for PCR Enzymes. For customized solutions please contact us.

Made in Denmark

Issued 09/2024