AMPLIQON
PCR ENZYMES & REAGENTS

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MADE IN **DENMARK**

AQ97 HiFi 2x Master Mix

1.5 mM $MgCl_2$ final concentration

Cat. No.	Reactions (50 µl)	AQ97 HiFi 2x Master Mix ID: 5500470
A770201	100	2 x 1.25 ml
A770203	500	10 x 1.25 ml
A770206	2500	50 x 1.25 ml
A770207	5000	25 x 5 ml
A770299 *	Sample - 20	0.5 ml

*1 ml Betaine Enhancer 5 M (ID: 5400000) is also included.

Contents

All-in-one 2x master mix containing the AQ97 High Fidelity DNA Polymerase, optimized Buffer, dNTPs and $MgCl_2$. 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 $MgCl_2$	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_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.
** 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[a)] 15 – 30 sec[b)] 10 – 60 sec[c)]	98 °C 55 – 70 °C 72 °C
1	5 minutes	72 °C

a. 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.

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 HiFi 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 Enhancer Solution often improves reaction performance. For ordering information visit 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 Enhancer 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.

$MgCl_2$

- The optimal $MgCl_2$ 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 or ask for our complete product list for PCR Enzymes. For customized solutions please contact us.

Made in Denmark

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