AMPLIQON
PCR ENZYMES & REAGENTS

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AQ97 HiFi Hot Start 2x Master Mix

2.0 mM $MgCl_2$ final concentration

MADE IN **DENMARK**

Cat. No.	Reactions (50 µl)	AQ97 HiFi Hot Start 2x Master Mix ID: 5500570
A790901	100	2 x 1.25 ml
A790903	500	10 x 1.25 ml
A790906	2500	50 x 1.25 ml
A790907	5000	25 x 5 ml
A790999	Sample - 20	0.5 ml

Contents

All-in-one 2x master mix containing the AQ97 High Fidelity Hot Start 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

- AQ97 HiFi Hot Start 2x Master Mix is not frozen at -20 °C. Simply mix the tube thoroughly and it is ready for preparation of the reaction mix.

1. 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 – 5 µl)	2.0 mM (2.0 – 4.5 mM)
Betaine (5 M)**	0 - 5 µl	0 – 0.5 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. See section *Strategies for Optimization*.

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

3. Three-step PCR program

Table 2: Recommended cycling conditions

Step	Duration of cycle	Temperature
Initial denaturation	30 sec – 2 min	98 °C
25 – 35 cycles	10 – 20 sec[a)] 15 – 30 sec[b)] 10 – 60 sec[c)]	98 °C 55 – 70 °C 72 °C
Final elongation	5 min	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 Hot Start 2x Master Mix, annealing temperature will likely be higher than with more traditional PCR buffers.**

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 plasmids) or short complex targets (< 3 kb). 30-60 seconds per kb is recommended for long amplicons (> 3 kb).**

4. Two-step PCR program

For targets with annealing temperatures ≥ 72°C (usually GC-rich primers), a 2-step thermocycling protocol (combining annealing and extension into one step) can be used.

Strategies for Optimization

High-yield/Nonspecific amplification

- Decreased primer concentration can improve PCR product specificity of long amplicons.
- Decreased extension time can improve PCR product specificity of short amplicons.
- Decreased PCR cycles will decrease PCR product yield.

Long-range amplification

- Longer extension times often resolve low-yield amplification of long amplicons.
- Increased template concentration will increase product yield.
- Increased number of PCR cycles will increase product yield.
- Increased primer concentration can increase product yield for some reactions.

GC-rich amplification

- The addition of 0.5 M Betaine Enhancer Solution often improves reaction performance.
- 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 concentration of 2.0 mM, as provided in this Master Mix (1x), will produce satisfactory results.

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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

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