AMPLIQON
PCR ENZYMES & REAGENTS

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AQ97 Hot Start High Fidelity DNA Polymerase

Concentration: 2 units/µl

Cat. No.	Units	AQ97 Hot Start High Fidelity DNA Polymerase ID: 5500350	5x AQ97 Buffer, 7.5 mM $MgCl_2$ ID: 5600650	$MgCl_2$ 25 mM ID: 5575801
A786799*	Sample – 40	20 µl	1.5 ml	1.5 ml

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

Contents

- **2 U/µl AQ97 Hot Start HiFi DNA Polymerase**
  50 mM Tris-HCl pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1.0 mM DTT, 0.1% Tween® 20, 50% Glycerol
- **5x AQ97 Buffer (7.5 mM $MgCl_2$)**
- **25 mM $MgCl_2$**
  For optional adjustment of Mg^{2+}.

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

- Allow all components to reach room temperature. A precipitate is often seen in the 5x AQ97 Buffer after thawing. It is recommended to completely thaw and thoroughly mix the buffer to ensure proper resuspension of precipitates.
- Amplification of templates with high GC content, extensive secondary structures as well as long range amplification may require more optimization - for tips see section *Strategies for Optimization*.

1. Prepare a reaction mix according to the following table.

Table 1: Recommended reaction setup

Component	Vol./reaction*	Final concentration*
5x AQ97 Buffer	5 µl	1x
dNTP mix (10 mM each)	0.5 µl	0.2 mM of each dNTP
Primer A (10 µM)	0.5 µl	0.2 µM
Primer B (10 µM)	0.5 µl	0.2 µM
25 mM $MgCl_2$	0 µl (0 – 3 µl)	1.5 mM (1.5 – 4.5 mM)
AQ97 Hot Start HiFi DNA Pol. 2U/µl	0.25 µl (0.125 – 0.5 µl)	0.5 unit (0.25 – 1 unit)
Betaine (5M)**	5 - 10 µl	1 - 2M
PCR-grade H_2O	X µl	-
Template DNA	X µl	genomic DNA: 20 ng (10 – 500 ng) plasmid DNA: 0.5 ng (0.1 – 1 ng) bacterial DNA: 5 ng (1 – 10 ng)
TOTAL volume	25 µl	-

* Suggested starting conditions; theoretically used conditions in brackets.
** Suggested for GC-rich amplification and long-range amplification. See section *Strategies for Optimization*.

2. Transfer the appropriate volume to a 96-well plate or strip compatible with the chosen thermal cycler. Seal the plate / strip.

3. Set up PCR program using the following guidelines:

Table 2: Recommended cycling conditions

Step	Duration of cycle	Temperature
Initial denaturation	2 min[a)]	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: 2 min initial denaturation is needed to fully activate AQ97 HS and sufficiently melt 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 1x AQ97 Buffer, 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).**

Strategies for Optimization

Long-range amplification

- Longer extension times often resolve low-yield amplification of long amplicons.
- Increased template concentration will increase product yield.
- The addition of 1-2 M Betaine Enhancer Solution often improves reaction performance (See Product Number A351104).
- Nonspecific amplification can often be alleviated with decreased primer concentrations. This may come at the cost of decreased yield.
- Decreased primer concentrations can improve amplification.
- Decreased extension time can improve specificity of short amplicons.

GC-rich amplification

- The addition of 1-2 M Betaine Enhancer Solution often improves reaction performance (See Product Number A351104).

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 concentration of 1.5 mM, as provided in the common 1x AQ97 Buffer, 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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