AMPLIQON
PCR ENZYMES & REAGENTS

Ampliqon A/S
www.ampliqon.com
info@ampliqon.com

Stenhuggervej 22
5230 Odense M
Denmark

Phone: +45 70201169
VAT No.: DK 26388899

# AQ97 Hot Start High Fidelity DNA Polymerase 2X Master Mix

**1.5 mM $MgCl_2$ final Concentration**



**Cat. No.: A790899**
**20 Reactions**

MADE IN **DENMARK**

| - | AQ97 Hot Start HiFi 2x Master Mix 3 mM $MgCl_2$ | Betaine Enhancer Solution 5 M |
|---|---|---|
| **ID No.** | **5500550** | **5400000** |
| **Cap colour** | **Red** | **White** |
| **Content** | 1 x 0.5 ml | 1 ml |

## Contents

All-in-one 2x hot start master mix recommended for low-bias, high fidelity amplification.

- AQ97 Hot Start HiFi 2x Master Mix: Optimized buffer, AQ97 Hot Start HiFi DNA Polymerase, dNTPs and $MgCl_2$.

## 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. It is recommended to completely thaw and thoroughly mix the master mix 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. 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 – 2M |
| PCR-grade $H_2O$ | X µl | - |
| Template DNA | X µl | genomic DNA: 50 ng (10 – 500 ng)<br>plasmid DNA: 0.5 ng (0.1 – 1 ng)<br>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*.

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)]<br>15 – 30 sec[b)]<br>10 – 60 sec[c)] | 98 °C<br>55 – 70 °C<br>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 AQ97 HS HiFi 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).**

## 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 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 extension time can improve specificity of short amplicons.
- Decreased primer concentrations can improve amplification.
- For amplicons longer than 11 kb, consider using AQ97 Hot Start HiFi (see A767501 for 100 units pack size)

### GC-rich amplification

- The addition of 1 - 2 M Betaine 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 this Master Mix (1x), will produce satisfactory results.



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**

Issued 02/2024