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AQ97 HiFi DNA Polymerase

Component	Cat#	A767501	A767503	A767504	A767504	Colour code of cap
AQ97 HiFi DNA Polymerase for long range PCR or PCR of difficult targets.		100 units 1x 50µL	500 units 1x 250µL	1000 units 2x 250µL	5000 units 5x 250µL	Black
5X AQ97 PCR Buffer		2x 1.5mL	4x 1.5mL	8 x 1.5mL	18 x 1.5mL	Clear
25mM MgCl2		1x 1.5mL	1x 1.5mL	2 x 1.5mL	15 x 1.5mL	Clear

Product Description

AQ97 High Fidelity DNA Polymerase is a thermostable, chimeric DNA Polymerase created specifically for low-bias, high fidelity amplification of a vast range of amplicons. AQ97 High Fidelity DNA Polymerase delivers high-speed elongation and processivity, due to its fusion with a DNA-binding domain.

Product Specifications

Concentration:	2 units/µL
Extension rate:	Min. 6 kb/min. at 72°C
5'-3' exonuclease activity:	Yes
Extra addition of A:	No
3'-5' exonuclease activity:	Yes
Nuclease contamination:	No
Protease contamination:	No
RNase contamination:	No
Self-priming activity:	No

Unit definition

One unit of AQ97 HiFi DNA Polymerase is defined as the amount of enzyme that incorporates 10nmol of dNTPs into acid-insoluble fraction in 30 minutes at 72°C under standard assay conditions.

Quality Control

Amplification efficiency: Amplification efficiency is tested in parallel amplification reactions and additionally against competitor products.
PCR reproducibility: PCR reproducibility is tested in parallel amplification reaction.
Exonuclease activity: Linearized DNA is incubated with AQ97 HiFi DNA Polymerase.
Endonuclease activity: Plasmid DNA is incubated with AQ97 HiFi DNA Polymerase.
RNase activity: RNA is incubated with AQ97 HiFi DNA Polymerase.
Protease activity: AQ97 HiFi DNA Polymerase is incubated in storage buffer.
Self-priming activity: PCR is performed under standard conditions, without primers, using AQ97 HiFi DNA Polymerase and human genomic DNA.

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Key Features

- Convenient reaction set-up
- High fidelity: >60x Taq[1)]
- Long range amplification: 11 kb for gDNA
- High elongation rate: 10 sec/kb
- Excellent performance on a vast range of amplicons (e.g., high AT and high GC)
- Recommended for cloning, mutagenesis and other molecular applications requiring extremely high fidelity

Application

Long range PCR
Detection of difficult to amply targets
Amplification of targets with high GC content
Cloning, mutagenesis and other molecular applications requiring extremely high fidelity

Supplied buffers/solutions

- 5X PCR optimized buffer

Stability

The Genaxxon bioscience AQ97 HiFi DNA Polymerase is shipped on wet ice but retain full activity at RT (+15°C to +25°C) for at least 1 week.

The Genaxxon bioscience AQ97 HiFi DNA Polymerase, including buffers and reagents, should be stored immediately upon receipt at -20°C. When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see tube label) without showing any reduction in performance. The Genaxxon bioscience AQ97 HiFi DNA Polymerase can also be stored at +2°C to +8°C up to 6 months.

Product Use Limitations

AQ97 HiFi DNA Polymerase is developed, designed, and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@genaxxon.com).

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PCR Protocol Part

Protocol using AQ97 HiFi DNA Polymerase

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions such as incubation times, temperatures and amount of template DNA may vary and must be determined individually. Amplification of templates with high GC content, high secondary structures as well as long range amplification may require more optimization – for tips see section Strategies for Optimization

Important notes before getting started
Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Points to do before starting

- If required, prepare a dNTP-mix containing 10mM of each dNTP (or ready-to-use product M3016 from Genaxxon bioscience). Store this mix in aliquots at -20°C.

Procedure

1. **Thaw 5X buffer, dNTPs or dNTP-mix and primer solutions at RT or on ice.**
   Keep solutions on ice after complete thawing and mix well before use to avoid local differences in salt concentrations.

   Optional: Prepare a primer mix of an appropriate concentration using sterile, nuclease-free water. This is recommended if several amplification reactions using the same primer pair are to be performed. The final volume of diluted primer mix plus the template DNA, added at step 4, should not exceed 12.5µL per reaction.

2. **Prepare a master mix according to Table 1**
   A master mix typically contains all the components needed for PCR except the template DNA.
   Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should always be included in every experiment.
   Keep this master mix on ice.

   Note: The Mg2+ concentration provided by the supplied AQ97 PCR-buffer will produce satisfactory results in most cases. However, in some cases, reactions may be improved by increasing the final Mg2+ concentration.

   Note: A negative control (PCR without template DNA) should be included in every experiment.

   Note: It is recommended that the PCR tubes are kept on ice until they are placed in the thermal cycler.

Table 1: PCR reaction components (**25µL PCR reaction**)

Components	Quantities for a 25µL PCR reaction
AQ97 HiFi DNA Polymerase (2U/µL)	0.25µL (0.5 units) [0.125 – 0.5µL (0.25 – 1 units)]
5X AQ97 PCR buffer	5µL
Nucleotides	0.5µL of 10mM ready-to-use dNTP mix (0.2mM of each dNTP).
25mM MgCl2 (optional)	0µL – 1.5mM (optional: 0-3µL – 1.5 – 4.5mM)
primer 1 (10µM):	0.5µL (0.2µM)
primer 2 (10µM):	0.5µL (0.2µM)
Probe (10µM) ** optional	x µL (0.05 – 1µM)
Template DNA	<500ng genomic DNA - <10ng bacterial DNA - <1ng plasmid DNA
Nuclease-free water	up to 25µL

* Suggested starting conditions. Theoretically used conditions in brackets.

** The necessary concentration of probe depends very much on the probe sequence and the kind of probe. Please test for optimum!

3. **Program the thermal cycler** according to the manufacturer's instructions.
   A typical PCR cycling program is outlined in Table 2. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

4. **Place PCR tubes in the thermal cycler and start program.**

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Table 2: PCR conditions (**Thermal cycler**)

Step	time	temperature	comments
Initial denaturation:	2 min.	98°C	
3-step cycling			
Denaturation:	10 20 sec. [a]	98°C	
Annealing:	15 30 sec. [b]	55 - 70°C	Approximately 5°C below Tm of primers.
Extension:	10 60 sec. [c]	72°C	For PCR products longer than 1kb, use an extension time of approximately 1min./kb DNA.
Final elongation	5 min	72°C	
Number of Cycles	25 - 35		
Final extension	5 min.		

Note: After amplification, samples can be stored at +2°C to +8°C overnight, or -20°C for long term storage.

a) Denaturation: 2 min initial denaturation is sufficient for 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 Tm (melting temperature) of the primers used. Because of the high salt content within the AQ97 High Fidelity DNA Polymerase 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 (<3kb). 30-60 seconds per kb is recommended for long amplicons (>3kb).

Strategies for Optimization:

Long-range amplification

- Longer extension times often resolve low-yield amplification of long amplicons.
- Increased amounts of AQ97 HiFi DNA Polymerase (up to 1 unit per 25µL PCR reaction) have often resolved low-yield reactions from very low targets (>8 kb).
- Increased dNTP concentration (up to 1.6µM) often increases yield and decreases unspecific product creation.
- The addition of 1-2 M B-enhancer solution often improves reaction performance.
- 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 B-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

https://primer3plus.com/cgi-bin/dev/primer3plus.cgi can be used to design primers.

MgCl2

- The optimal MgCl2 concentration should be determined empirically, but in most cases a final concentration of 1.5mM, as provided in AQ97 High Fidelity DNA Polymerase 5X PCR Buffer, will produce satisfactory results.

Table 3 provides the volume of 25 mM MgCl2 to be added to the master mix if a higher MgCl2 concentration is required.

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Table 3b: Final MgCl2 concentration in a **25µL reaction**

Final MgCl2 conc. In PCR reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25mM MgCl2 per 25µL reaction (µL)	3	4	5	6	7	8	9

Note: The optimal Mg2+ concentration should be determined empirically but in most cases a concentration of 1.5mM, as provided in the AQ97 HiFi DNA Polymerase 5X Buffer will produce satisfactory results.

Table 4: Recommendations for Standard PCR-Primers

Length:	18-30 nucleotides
GC-Content:	40-60%
Tm:	Design primer pairs with similar Tm values.
	Optimal annealing temperature may be above OR below the estimated Tm. As a starting point, use an annealing temperature of 3°C to 5°C below Tm of the primer with the lower Tm-Value.
Sequence:	Avoid complementarities of two or more bases at the 3' ends of primer pairs.
	Avoid runs of 3 or more Gs or Cs at the 3' end.
	Avoid a 3'-end T.
	Avoid complementary sequences within primer and between primer pairs.

Table 5: Migration Chart of some Gel Tracking Dyes

Dye in agarose gel	0.5%-1.5%	2.0%-3.0%	CAS-number	Cat-No. Genaxxon
Xylene cyanol	10000bp - 4000bp	750bp - 200bp	2650-17-1	M3312
Cresol Red	2000bp - 1000bp	200bp - 125bp	62625-29-0	M3371
Bromophenol blue	500bp - 400bp	150bp - 50bp	115-39-9	M3092
Orange G	<100bp	<20bp	1936-15-8	M3180
Tartrazine	<20bp	<20bp	1934-21-0	

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