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AQ97 2X PCR Master Mix

with *High Fidelity DNA Polymerase*

	Cat#	A770101	A770103	A770106	Colour code of cap
Component					
Premixed 2-time PCR Mastermix with High Fidelity DNA Polymerase for long range PCR or PCR of difficult targets.		2x 1.25mL for 200x 25µL PCR reactions	10x 1.25mL for 1000x 25µL PCR reactions	50x 1.25mL for 5000x 25µL PCR reactions	blue

Product Description

AQ97 High Fidelity DNA Polymerase 2x Master Mix is an all-in-one 2x master mix containing the AQ97 High Fidelity DNA Polymerase, AQ97 Buffer, dNTPs and MgCl2. Simply mix AQ97 High Fidelity DNA Polymerase 2x Master Mix with primers, DNA template and water and you are ready to carry out PCR.

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 time ready-to-use PCR master mix
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

Quality Control

Amplification efficiency: Amplification efficiency is tested in parallel amplification reactions and additionally against competitors products.
PCR reproducibility: PCR reproducibility is tested in parallel amplification reaction.
Exonuclease activity: Linearized DNA is incubated with AQ97 PCR Mastermix (2X).
Endonuclease activity: Plasmid DNA is incubated with AQ97 PCR Mastermix (2X).
RNase activity: RNA is incubated with AQ97 PCR Mastermix (2X).
Protease activity: AQ97 PCR Mastermix (2X) is incubated in storage buffer.
Self-priming activity: PCR is performed under standard conditions, without primers, using AQ97 PCR Mastermix (2X) 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

Stability

The Genaxxon bioscience AQ97 PCR Mastermix (2X) 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 PCR Mastermix (2X), 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 PCR Mastermix (2X) can also be stored at +2°C to +8°C up to 6 months.

Product Use Limitations

AQ97 PCR Mastermix (2X) 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 PCR Mastermix (2X)

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.

Procedure

1. **Thaw primer solutions**
   Keep on ice after complete thawing and mix well before use.

   Optional: Prepare a primer mix of an appropriate concentration using sterile, demineralised 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 25µL per reaction.
2. **Thaw AQ97 High Fidelity DNA Polymerase 2x Master Mix**
   Note: It is recommended to completely thaw and thoroughly mix the master mix to ensure proper resuspension of precipitates.
3. **Distribute the appropriate volume of diluted primer mix into the PCR tubes containing the** AQ97 PCR Mastermix (2X)
4. **Add template DNA** to individual tubes containing the reaction mix (<1µg/reaction) to the individual PCR tubes.
5. A negative control (PCR without template DNA) should be included in every experiment. It is recommended that the PCR tubes are kept on ice until they are placed in the thermal cycler.

Table 1a: PCR reaction components using AQ97 PCR Mastermix (2X) **(25µL PCR reaction)**

Components	Quantities
AQ97 PCR Mastermix (2X)	12.5µL
primer 1 (10µM):	variable volume: 0.1 - 0.25µM (5 - 12 pmol absolute)
primer 2 (10µM):	variable volume: 0.1 - 0.25µM (5 - 12 pmol absolute)
25mM MgCl2 (optional)	0µL - 3µL (1.5mM - 4.5mM)
B-enhancer solution (optional) **	5µL - 10µL (1 - 2M)
Template DNA	variable volume: <0.5ng plasmid DNA (0.1ng - 1ng) variable volume: <50ng genomic DNA (10ng - 500ng) variable volume: 5ng bacterial DNA (1ng - 10ng)
Nuclease-free water	up to 25µL

Table 1b: PCR reaction components using AQ97 PCR Mastermix (2X) **(50µL PCR reaction)**

Components	Quantities
AQ97 PCR Mastermix (2X)	25µL
primer 1 (10µM):	variable volume: 0.1 - 0.25µM (5 - 12 pmol absolute)
primer 2 (10µM):	variable volume: 0.1 - 0.25µM (5 - 12 pmol absolute)
25mM MgCl2 (optional)	0µL - 6µL (1.5mM - 4.5mM)
B-enhancer solution (optional) **	10µL - 20µL (1 - 2
Template DNA	variable volume: <0.5ng plasmid DNA (0.1ng - 1ng) variable volume: <50ng genomic DNA (10ng - 500ng) variable volume: 5ng bacterial DNA (1ng - 10ng)
Nuclease-free water	up to 50µL

6. **When using a thermal cycler with a heated lid**, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50µL mineral oil.

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7. **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.
8. **Place PCR tubes in the thermal cycler and start program.**

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.
Number of Cycles	25 - 35	72°C	
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.
- 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 2x Master Mix, 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 3a: Final MgCl2 concentration in a **50µL 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 50µL reaction (µL)	0	1	2	3	4	5	6

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 PCR Mastermix (2X) 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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