AMPLIQON
PCR ENZYMES & REAGENTS

AQ90 High Fidelity DNA Polymerase

With 10x AQ90 Buffer (20 mM $MgCl_2$)

Concentration: 2 units/µl

SAMPLE

Cat. No.: A456699
40 Units

-	AQ90 High Fidelity DNA Polymerase	10x AQ90 Buffer, 20 mM $MgCl_2$	$MgCl_2$ 25 mM	Betaine Enhancer Solution
ID No.	**5500200**	**5600600**	**5575801**	**5400000**
Cap colour	**Green**	**Red**	**Clear**	**White**
Content	20 µl	1.5 ml	1.5 ml	1 ml

MADE IN **DENMARK**

Key Features

- High fidelity – measured up to 50x *Taq* fidelity
- Excellent coverage for amplification of difficult amplicons with low to high GC content
- Long range capability: 8.5 kb for gDNA and ≤ 12.5 kb for λDNA
- Recommended for cloning, mutagenesis and other molecular applications requiring extremely high fidelity

AQ90 High Fidelity DNA Polymerase is a thermostable DNA Polymerase with proofreading ability. AQ90 High Fidelity DNA Polymerase exhibits 5'→3' DNA polymerase activity and 3'→5' proofreading exonuclease activity. The latter allows the enzyme to correct misincorporated nucleotides. By using the 10x AQ90 Buffer the polymerase exhibits robust amplification of targets with low to high GC content, as well as a fidelity* up to 50x *Taq*.
*Estimated by NGS technology using the Illumina MiSeq instrument.

Protocol

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.

Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis. **Work on ice at all times.**

1. Thaw 10x AQ90 Buffer, dNTP mix and primer solutions. **A precipitate is often seen in the 10x AQ90 Buffer after thawing. It is recommended to completely thaw and thoroughly mix the buffer to ensure proper resuspension of precipitates.**

2. Prepare a master mix according to Table 1. The master mix typically contains all the components needed for amplification except the template DNA. It is important to add AQ90 High Fidelity DNA Polymerase last in order to prevent primer degradation caused by the 3'→5' exonuclease activity.

Table 1. Reaction components

Component	Vol./reaction*	Final concentration*
10x AQ90 Buffer	2.5 µl	1x
dNTP mix (12.5 mM each)	0.4 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)	2 mM (2 – 5 mM)
AQ90 HiFi DNA Pol. 2U/µl	0.25 µl (0.125 – 0.5 µl)	0.5 units (0.25 – 1 units)
Betaine (5M) (optional)	5 - 10 µl	1 - 2M
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	25 µl	-

* Suggested starting conditions; theoretically used conditions in brackets.

3. Mix the master mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, e.g. by pipetting the master mix up and down a few times.

4. Add template DNA to the individual tubes containing the master mix.

5. Program the thermal cycler according to the manufacturer's instructions. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

6. Place the tubes in the thermal cycler and start the reaction.

Table 2. Three-step PCR program

Cycles	Duration of cycle	Temperature
1	2 – 5 minutes[a]	95 °C
25 - 35	10 – 30 seconds[b] 20 – 40 seconds[c] 30 seconds[d]	95 °C 55 – 70 °C 72 °C
1	5 minutes[e]	72 °C

[a] Initial denaturation step (optional).

[b] Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 10 – 30 seconds. It causes melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

[c] Annealing step: The reaction temperature is lowered to 55 – 70 °C for 20 – 40 seconds allowing annealing of the primers to the single-stranded DNA template. 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 10x AQ90 Buffer, annealing temperature will likely be higher than with more traditional PCR buffers.**

[d] Extension/elongation step: AQ90 High Fidelity DNA polymerase has its optimum activity temperature at 72 °C. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand. The extension time depends on the length of the DNA fragment to be amplified. **Generally, we recommend an extension time of 1 minute per kb, especially for longer amplicons.**

[e] Final elongation: This single step is occasionally performed at a temperature of 72 °C for 2 - 5 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

Notes for optimization of PCR conditions:

- The optimal $MgCl_2$ concentration should be determined empirically but in most cases a concentration of 2 mM, as provided in the common 1x AQ90 Buffer, will produce satisfactory results. Table 3 provides the volume of 25 mM $MgCl_2$ to be added to the master mix if a higher $MgCl_2$ concentration is required.

SAMPLE

Table 3. Additional volume (µl) of $MgCl_2$ per 25 µl reaction

Final $MgCl_2$ conc. in reaction (mM)	2.0	2.5	3.0	3.5	4.0	4.5	5
Volume of 25 mM $MgCl_2$	0	0.5	1	1.5	2	2.5	3

- For difficult amplicons, such as GC-rich templates, those with secondary structures or very long amplicons the addition of 1 - 2 M Betaine solution might improve reaction performance (See Additional Products for ordering information). 5 - 10 % DMSO can also be used to improve reaction performance. If using high concentrations of DMSO the annealing temperature has to be lowered as it decreases the primer T_m.

- 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.

Kit Components

- **2U/µl AQ90 High Fidelity DNA Polymerase in Storage Buffer**
  50 mM Tris-HCl pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1.0 mM DTT, 0.1% Tween® 20, 50% Glycerol

- **10x AQ90 Buffer (20 mM $MgCl_2$)**

- **5 M Betaine Enhancer Solution**

- **25 mM $MgCl_2$**
  For optimization of PCR conditions if required.

Recommended Storage and Stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.
Optional: Store at +4 °C for up to 6 months.

Quality Control

AQ90 High Fidelity DNA Polymerase is tested for contaminating activities with no traces of endonuclease activity or nicking activity.

Unit Definition

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into an acid-precipitable form of DNA in 30 minutes at 72 °C under standard assay conditions.

Related Products

AQ90 High Fidelity DNA Polymerase 2 U/µl	Cat. No.
With 10x AQ90 Buffer – 100 U	**A457401**
With 10x AQ90 Buffer – 500 U	**A457403**
With 10x AQ90 Buffer – 1000 U	**A457404**
With 10x AQ90 Buffer – 2500 U	**A457406**

AQ90 High Fidelity DNA Polymerase 2x Master Mix	Cat. No.
100 reactions	**A470701**
500 reactions	**A470703**
2500 reactions	**A470706**
5000 reactions	**A470707**

PCR Grade Water	Cat. No.
6 x 5 ml	**A360056**

Betaine Enhancer Solution 5 M	Cat. No.
5 x 1 ml	**A351104**

For Research Use Only. Not for use in diagnostics procedures.

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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