AMPLIQON
PCR ENZYMES & REAGENTS

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AccuPOL DNA Polymerase

Concentration: 2.5 units/µl

MADE IN **DENMARK**

Cat. No.	Units	AccuPOL DNA Polymerase ID: 5101800
A210002	250	100 µl
A210003	500	200 µl
A210004	1000	2 x 200 µl
A210006	2500	5 x 200 µl

Key Features

AccuPOL DNA Polymerase is a thermostable enzyme with proof-reading ability, which can be used in primer extension reactions and other molecular biology applications. AccuPOL exhibits 5'→3' DNA polymerase activity and 3'→5' proofreading exonuclease activity. The latter allows the enzyme to correct misincorporated nucleotides. AccuPOL has an error rate* of 1.1 x 10^{-6}, which gives a 16 x greater fidelity than Taq DNA Polymerase.
Optimal reaction conditions are achieved by using the 10x Ammonium buffer containing $MgCl_2$. AccuPOL DNA Polymerase is recommended for applications, which require high fidelity or blunt ending.
* The error rate is measured using the LacIOZ assay. Fidelity depends also on reaction conditions.

Kit Components

AccuPOL DNA Polymerase in Storage Buffer

2.5 U/µl AccuPOL DNA Polymerase, 50 mM Tris-HCl pH 8.5, 0.1 mM EDTA, 1.0 mM DTT, 0.1% Tween® 20, 50% Glycerol.

We recommend using the Ampliqon AccuPol DNA Polymerase with the Ampliqon Ammonium Buffer: Tris-HCl pH 8.5, $(NH_4)_2S0_4$, 15 mM $MgCl_2$, 1% Tween® 20, optional without $MgCl_2$ and/or Tween® 20.

Recommended Storage and Stability

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

Quality Control

AccuPOL 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 nmol of dNTPs into an acid-precipitable form of DNA in 30 minutes at 72 °C under standard assay conditions.

Protocol

This protocol serves as a guideline to ensure optimal PCR results when using AccuPOL DNA Polymerase. Optimal reaction conditions such as incubation times, temperatures and amount of template DNA may vary and must be determined individually.

1. Thaw 10x Buffer, dNTP mix, and primer solutions. **It is important to thaw the solutions completely (some buffers need to reach room temperature) and mix thoroughly before use to avoid localized concentrations of salts**. Keep all components on ice. The polymerase is provided in glycerol and does not need thawing. Keep it at -20 °C at all times.

2. Prepare a master mix according to Table 1. The master mix typically contains all the components needed for extension except the template DNA. We recommend Ampliqon Ammonium Buffer to be used with AccuPOL Polymerase.

   Important: It is critical to withhold AccuPOL Polymerase until after addition of dNTPs. Otherwise, the proofreading activity of the polymerase may degrade the primers resulting in non-specific amplification and reduced product yield.

Table 1. Reaction components (master mix and template DNA)

Component	Vol./reaction*	Final concentration*
10x Buffer	5 µl	1x
25 mM $MgCl_2$	3 µl (2 – 9 µl)	1.5 mM (1 – 4.5 mM)
dNTP mix (10 mM each)	1 µl	0.2 mM of each dNTP
Primer A (10 µM)	1 µl (0.5 – 5 µl)	0.2 µM (0.1 – 1.0 µM)
Primer B (10 µM)	1 µl (0.5 – 5 µl)	0.2 µM (0.1 – 1.0 µM)
AccuPol DNA Pol.	0.6 µl (0.6 – 2 µl)	1.5 units (1,5 – 5 units)
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	50 µl	-

* Suggested starting conditions; theoretically used conditions in brackets. The final volume can be reduced to 25 µl by using half of the volumes suggested in Vol./reaction, e.g. 0.3 µl AccuPol instead of 0.6 µl AccuPol.

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. **AccuPOL is a proofreading enzyme and requires an extension time of 1 – 2 min/kb.**
   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.

Three-step PCR program

Cycles	Duration of cycle	Temperature
1	1 – 2 minutes[a]	95 °C
25 - 35	30 – 60 seconds[b] 30 seconds[c] 1 – 4 minutes[d]	95 °C 50 – 65 °C 72 °C
1	5 minutes[e]	72 °C

[a.] Initial denaturation step.

[b.] Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 30 – 60 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 50 – 65 °C for 30 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.

[d.] Extension/elongation step: The extension rate of AccuPOL DNA polymerase is slower than that of Taq DNA Polymerase. Therefore, during the extension step, allow approximately 2 minutes for every 1kb to be amplified (minimum extension time of 1 minute).

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

Notes:

- For longer DNA targets more DNA polymerase could be added to the PCR master mix.

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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