AMPLIQON
PCR ENZYMES & REAGENTS

AccuPOL™ DNA Polymerase

With 10x Ammonium Buffer (Mg^{2+} free, Tween free)

Concentration: 2.5 units/µl

Cat. No.: A211502

A211502

Cat. No.	Units	AccuPOL DNA Polymerase	10x Ammonium Buffer, Mg^{2+} free, Tween free	$MgCl_2$ 25 mM
ID No. Cap colour	-	5101800 Blue	5101010 Black	5575801 Yellow
A211502	250	100 µl	1.5 ml	1.5 ml

Key Features

- High fidelity – Proofreading
- Processes up to 3 kb with extremely high fidelity
- Recommended for cloning or mutagenesis
- Renders blunt ended DNA

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 Polymerase.
Optimal reaction conditions are achieved by using the 10x Ammonium buffer containing $MgCl_2$. AccuPOL DNA Polymerase is recommended for applications, which require extremely high fidelity or blunt ending.

* The error rate is measured using the LacIOZ assay. Fidelity depends also on reaction conditions.

Kit Components

10x Ammonium Buffer, Mg^{2+} free, Tween free

Tris-HCl pH 8.5, $(NH_4)_2S0_4$.

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, 0.1% NP40, 50% Glycerol.

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, nicking activity or exonuclease 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.

Protocol

This protocol serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

Notes:

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis. Work on ice at all times.
- When using Mg^{2+} free buffers, the addition of $MgCl_2$ to the reaction is imperative because Mg^{2+} is required for polymerase activity. In most cases a concentration of 1.5 mM will produce satisfactory results. Use 25 mM $MgCl_2$ to adjust the Mg^{2+} concentration according to Table 1.

Table 1. Additional volume (µl) of $MgCl_2$ per 50 µl reaction

Final $MgCl_2$ conc. in reaction (mM)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Volume of 25 mM $MgCl_2$	1	2	3	4	5	6	7	8	9

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 2. 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 2. Reaction components (master mix and template DNA)

Component	Vol./reaction*	Final concentration*
10x Buffer	5 µl	1x
25 mM $MgCl_2$	3 µl (1 – 10 µl)	1.5 mM (0.5 – 5 mM)
dNTP mix (12.5 mM each)	0.8 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.	0.4 µl (0.4 – 2 µl)	1 unit (1 – 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, eg. 0.2 µl AccuPol instead of 0.4 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.

Related Products

Taq Polymerase (500 units) *	Cat. No.
Taq DNA Polymerase 5 U/µl	A110003
• with 10x Ammonium Buffer	A111103
• 5x PCR Buffer RED	A111803
Taq DNA Polymerase 5 U/µl, RED	A200003
• with 10x Ammonium Buffer	A201103
Taq DNA Polymerase 5 U/µl, glycerol free	A100003
• with 10x Ammonium Buffer	A101103

Hot Start Polymerase (500 units) *	Cat. No.
TEMPase Hot Start DNA Polymerase, 5 U/µl	A220003
• with 10x Ammonium Buffer	A221103
• 5x PCR Buffer RED	A221803
TEMPase Hot Start DNA Polymerase, glycerol free 5 U/µl	A240003
• with 10x Ammonium Buffer	A241103

High Fidelity - Proof reading (500 units) **	Cat. No.
AccuPOL DNA Polymerase 2.5 U/µl	A210003
• with 10x Ammonium Buffer	A211103

*Available in kits including one or two buffers (Ammonium Buffer, Standard Buffer or Combination Buffer). **AccuPOL only available in kits with Ammonium Buffer. All kits include extra 25 mM $MgCl_2$.

Buffers for DNA polymerases *	Cat. No.
10x Ammonium Buffer, 3 x 1.5 ml	A301103
10x Standard Buffer, 3 x 1.5 ml	A302103
10x Combination Buffer, 3 x 1.5 ml	A303103
5x PCR Buffer RED, 6 x 1,5 ml **	A301810

*Ammonium Buffer, Standard Buffer and Combination Buffer are also available as Mg^{2+} free buffers, detergent free buffers and Mg^{2+} and detergent free buffers. **For direct gel loading and visualisation.

Taq Master Mixes (500 x 50 µl reactions) *	Cat. No.
2x Master Mix, 1.5 mM $MgCl_2$ final concentration	A140303
2x Master Mix RED, 1.5 mM $MgCl_2$ final concentration	A180303

TEMPase Hot Start Master Mixes (500 x 50 µl reactions) *	Cat. No.
2x Master Mix A**, 1.5 mM $MgCl_2$ final concentration	A230303
2x Master Mix A**BLUE, 1.5 mM $MgCl_2$ final concentration	A290403

*Master mixes available also in 1.1x variants as well as 2 mM $MgCl_2$ variants, **Mix A is Ammonium Buffer based, also available as Mix C based on Combination Buffer.

Special Master Mixes (500 x 50 µl reactions)	Cat. No.
Multiplex 2x Master Mix, 3 mM $MgCl_2$ final concentration	A260303
GC TEMPase 2x Master Mix I – for GC-rich templates	A331703
GC TEMPase 2x Master Mix II – for GC-rich templates	A332703

Real-time PCR Master Mixes (400 x 25 µl reactions)	Cat. No.
RealQ Plus 2x Master Mix for probe,	
• without ROX™	A313402
• with low ROX™	A314402
• with high ROX™	A315402
RealQ Plus 2x Master Mix Green	
• without ROX™	A323402
• with low ROX™	A324402
• with high ROX™	A325402

Ultrapure dNTPs*	Cat. No.
dNTP Mix 40 mM (2 x 500 µl): 10 mM each dA, dC, dG, dT	A502004
dNTP Set, 100 mM each: 250 µl of each dA, dC, dG and dT	A511104

*Other concentrations and Single dNTPs are available.

Loading Buffers and Ladders	Cat. No.
5x Loading Buffer Red *, 5 x 1 ml	A608104
PCR DNA Ladder **, 100 – 3000 bp, 1 x 0.5 ml	A610341

* Also available with Blue, Orange or Cyan. ** Available in different size ranges.

Reagents for *in vitro* laboratory use only.

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 02/2017

Ampliqon A/S, Stenhuggervej 22, DK-5230 Odense M, Denmark. Phone: +45 70201169 Fax: +45 70201179 info@ampliqon.com www.ampliqon.com