

AccuPOL™ DNA Polymerase

With 10x Ammonium Buffer (Mg²⁺ free, Tween free)

Concentration: 2.5 units/μl



Cat. No.: A211504

A211504

| Cat. No. | Units | AccuPOL DNA Polymerase | 10x Ammonium Buffer, Mg ²⁺ free, Tween free | MgCl ₂ 25 mM |
|------------|-------|------------------------|--|-------------------------|
| ID No. | - | 5101800 | 5101010 | 5575801 |
| Cap colour | - | Blue | Black | Yellow |
| A211504 | 1000 | 2 x 200 μl | 2 x 1.5 ml | 2 x 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 proofreading 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 MgCl₂. 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, Mg²⁺ free, Tween free

Tris-HCl pH 8.5, (NH₄)₂SO₄.

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 Mg²⁺ free buffers, the addition of MgCl₂ to the reaction is imperative because Mg²⁺ is required for polymerase activity. In most cases a concentration of 1.5 mM will produce satisfactory results. Use 25 mM MgCl₂ to adjust the Mg²⁺ concentration according to Table 1.

Table 1. Additional volume (μl) of MgCl₂ 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 ₂ O | 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).

^e 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 MgCl₂.

| 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 Mg²⁺ free buffers, detergent free buffers and Mg²⁺ 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 MgCl₂ 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
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