

AccuPOL DNA Polymerase

With 10 x Ammonium Buffer
(Mg Free and Tween Free)

Concentration: 2.5 units/μL

Catalogue no.: 210406 (2500 Units)

Cat. No.	Size Units	10X Ammonium Buffer Mg Free (Tween Free)	MgCl ₂ 25 mM
210402	250	1.5 mL	1.5 mL
210403	500	1.5 mL	1.5 mL
210404	1000	2 x 1.5 mL	2 x 1.5 mL
210406	2500	4 x 1.5 mL	4 x 1.5 mL

Store at -20°C. Reagent for in-vitro laboratory use only

General Description

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 both 5'→3' DNA polymerase activity and 3'→5' proofreading exonuclease activity. It is recommended for applications, which require extremely high fidelity or blunt ending.

Optimal reaction conditions are achieved by using the 10x Ammonium buffer provided with the enzyme. 25 mM MgCl₂ is also included separately.

Key Features

- Provides higher fidelity than Taq DNA Polymerase
- Produces blunt-ended fragments
- Processes <3 kb with extremely high fidelity

Unit Definition

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

10X Ammonium Reaction Buffer

Tris-HCl pH 8.5, (NH₄)₂SO₄,

AccuPOL Storage Buffer

50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol, 0.1% NP40, 0.1% Tween-20.

Suggested Protocol using AccuPOL DNA Polymerase

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 individually determined.

Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis.

1. Thaw 10X Ammonium Buffer (Mg Free Tween Free), dNTP mix, and primer solutions. **It is important to mix the solutions completely before use to avoid localized concentrations of salts.**
2. Prepare a master mix according to Table 1. The master mix typically contains all the components needed for extension except the template DNA.

The optimal MgCl₂ concentration should be determined empirically but in most cases a concentration of 1.5 mM, in the 1X Ammonium Buffer Mg Free Tween Free, will produce satisfactory results. Table 2 provides the volume of 25 mM MgCl₂ to add to the master mix for the required MgCl₂ concentration.

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

Important note: 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.

Component	Vol./reaction	Final Conc.
10X Ammonium Buffer Mg Free Tween free	5 μL	1X
MgCl ₂ (25 mM)	Variable	See Table 2
dNTP mix (12.5 mM of each)	0.8 μL	0.2 mM of each dNTP
Primer A	Variable	0.1–0.5 μM
Primer B	Variable	0.1–0.5 μM
AccuPOL DNA Polymerase	1 μL	2.5 units per reaction
Distilled Water	Variable	----
Template DNA	Variable	0.1–0.5 μg per reaction
TOTAL volume	50 μL	----

Table 2. MgCl₂ concentration in a 50 μL reaction

Final MgCl ₂ conc. in reaction (mM)	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Additional volume of 25 mM MgCl ₂ per reaction (μL):	1	2	3	4	5	6	7

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 (0.1–0.5 μg/reaction) to the individual tubes containing the master mix.
5. Program the thermal cycler according to the manufacturer's instructions.

Notes:

- AccuPOL is a proofreading enzyme and require 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 programme:

Cycles	Duration of cycle	Temperature
1	1 – 2 minutes ^a	95 °C
25-35	30 - 60 seconds ^b	95 °C
	30 seconds ^c	50-65 °C
	1 – 4 minutes ^d	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 DNA 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 degrees Celsius below the T_m of the primers used.

^d Extension/elongation step: AccuPOL DNA Polymerase is lower 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.

Quality Control

Endonuclease, exonuclease and priming activities are not detected after 3 hours incubation of 1 µg of pUC19 plasmid DNA and 0.5 µg EcoR I digested lambda phage DNA at 72°C in the presence of 40 units of AccuPOL DNA Polymerase.

Related Products

Description	Cat. no.
Taq DNA Polymerase (1000 units) Glycerol free	100103
Taq DNA Polymerase (500 units) with 10X Ammonium Reaction Buffer with 10X Standard Reaction Buffer	110303
Taq DNA Polymerase (500 units) with 10X Combination Buffer	110403
Taq DNA Polymerase (500 units) with 10X Mg ⁺⁺ Free Ammonium Buffer	110503
Taq DNA Polymerase 2.0X Master Mix (100 r) with 2.0 mM MgCl ₂	150301
Taq DNA Polymerase 2,0X MaMi RED (100 r) with 1.5 mM MgCl ₂ ,	180301
Taq DNA Polymerase 2.0X MaMi RED (100 r) with 2.0 mM MgCl ₂	190301
AccuPOL DNA Polymerase (500 units)	210303
TEMPase Hot Start DNA Polymerase (500 units) with 10X TEMPase Buffer I with 10X TEMPase Buffer II	220303
TEMPase Hot Start 2 x Master Mix with TEMPase Buffer I (100 r)	230301
TEMPase Hot Start 2 x Master Mix with TEMPase Buffer II (100 r)	230701
TEMPase Hot Start 2 x Master Mix Blue With TEMPase Buffer I (100 r)	230301
TEMPase Hot Start 2 x Master Mix Blue With TEMPase Buffer II (100 r)	230701
RealQ PCR 2 x Master Mix (200 reactions) for probe	250407
RealQ PCR 2 x Master Mix (200 reactions) With green dye	250507
dNTP Mix (2 x 500µl) (12.5 mM of each dA, dC, dG and dT)	501004
dNTP Mix, (2 x 500 µl) (10 mM of each dA, dC, dG and dT),	502004

(Other product sizes available)

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NOTICE

In certain countries, patents cover the PCR process. This product is intended for researchers having a license to perform PCR or those not required to obtain a license.

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