UniAccu *Pfu* DNA Polymerase (Mg^{2+} Plus Buffer)

Product Information

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Kit Contents

Components	ATR-P515-1 (250 Units)
UniAccu *Pfu* DNA Polymerase (2.5 U/µL)	50 µL
10X UniAccu *Pfu* Reaction Buffer (with $MgSO_4$)	1 mL

Product Description

UniAccu *Pfu* DNA Polymerase is a highly purified, recombinant enzyme derived from *Pyrococcus furiosus*, engineered for high-fidelity 5'→3' DNA synthesis. The enzyme exhibits robust 3'→5' exonuclease (proofreading) activity, enabling correction of nucleotide misincorporation errors, but lacks 5'→3' exonuclease and reverse transcriptase activities. With a fidelity approximately 50-fold higher than Taq DNA polymerase and 2-fold higher than standard *Pfu* DNA polymerase, UniAccu *Pfu* is the preferred choice for applications requiring exceptional accuracy in DNA amplification. The enzyme ensures reliable performance across diverse primer-template systems, surpassing the specificity and yield of conventional *Pfu* DNA polymerases from other suppliers. Each production lot undergoes stringent quality control, including assays for PCR specificity, reproducibility, and fidelity. UniAccu *Pfu* is optimized for high-fidelity amplification of DNA fragments up to 6 kb from plasmid, genomic, or cDNA templates, producing blunt-ended PCR products suitable for blunt-end cloning. Its robust performance supports applications such as site-directed mutagenesis, high-throughput PCR, and cDNA cloning.

Applications

- High-fidelity PCR and primer-extension reactions
- High-throughput PCR amplification
- Blunt-end cloning
- Site-directed mutagenesis
- Reverse transcription PCR (RT-PCR) for cDNA cloning and expression

Highlights

- High-yield and specific PCR products across diverse primer-template systems
- 50-fold higher fidelity compared to *Taq* DNA polymerase
- 2-fold higher fidelity compared to standard *Pfu* DNA polymerase
- Generates blunt-ended PCR products for seamless cloning
- Robust amplification of fragments up to 6 kb

Source

Recombinantly expressed and purified from an *Escherichia coli* strain harboring the cloned *Pfu* polymerase gene.

Unit Definition

One unit is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-insoluble material in 30 minutes at 72°C, using activated calf thymus DNA as the template.

Storage Buffer Composition

20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% (v/v) Tween 20, 0.5% (v/v) Nonidet P-40, 50% (v/v) glycerol.

10X UniAccu *Pfu* Reaction Buffer Composition

200 mM Tris-HCl (pH 8.8 at 25°C), 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 1% (v/v) Triton X-100, 1 mg/mL BSA.

Storage

Store all kit components at -20°C.

Shipping

The kit is shipped on gel ice packs.

Protocol

To minimize pipetting errors and ensure consistency, prepare a PCR master mix containing all components except UniAccu *Pfu* DNA Polymerase, which should be added last. Prepare sufficient master mix for the number of reactions plus one additional to account for pipetting variability.

1. Thaw all components on ice, gently vortex, and briefly centrifuge to collect contents.
2. In a sterile, thin-walled PCR tube on ice, assemble the following components for a 25 µL reaction (scalable to 50 µL if required):

Components	Reaction Volume	Final Concentration
10X UniAccu *Pfu* Reaction Buffer (with $MgSO_4$)	2.5 µL	1X
dNTP Mix (10 mM each)	0.5 µL	200 µM each
Forward Primer	0.5-2.5 µL	0.1-1.0 µM
Reverse Primer	0.5-2.5 µL	0.1-1.0 µM
Template DNA*	Variable	10 pg - 1 µg
UniAccu *Pfu* DNA Polymerase (2.5 U/µL)	0.5-1 µL	1.25–5 U
Nuclease-free Water	to 25 µL	-

***Recommended template DNA concentrations:**

Template DNA	Concentration
Animal & Plant Genomic DNA	0.1 - 1 µg
E. coli Genomic DNA	10 - 100 ng
cDNA	1–5 µL (≤10% of total PCR volume)
Plasmid DNA	0.1 - 10 ng
λDNA	0.5 - 10 ng

3. Gently vortex the reaction mix and briefly centrifuge.
4. If using a thermal cycler without a heated lid, overlay with 25 µL of mineral oil.
5. Perform PCR using the following thermal cycling conditions:

Step	Temperature (°C)	Time	Number of Cycles
Initial Denaturation [a]	95	1-3 min	1
Denaturation	95	30 sec	25-35
Annealing [b]	Tm-5	30 sec	
Extension [c]	72	2 min/kb	
Final extension	72	5-15 min	1

[a] Adjust initial denaturation (1–3 min for ≤50% GC content; 5–10 min for complex or GC-rich templates).

b Set annealing temperature 3–5°C below the primer's melting temperature (Tm). Optimize in 1–2°C increments for specificity.

[c] Use 2 min/kb at 72°C for amplicons ≤2 kb; for >2 kb, reduce to 68°C to prevent enzyme inactivation.

6. Load 3–5 µL of PCR product directly onto an agarose gel for analysis.

Important Considerations for Successful PCR

1. Contamination Prevention

To mitigate contamination risks during PCR, which can amplify trace contaminants:

- Perform DNA preparation, reaction setup, amplification, and analysis in physically separated areas.
- Use a laminar flow cabinet with UV sterilization for reaction setup.
- Wear clean gloves and use dedicated reagent containers and positive displacement pipettes or aerosol-resistant tips.
- Employ PCR-certified reagents and nuclease-free water.
- Include no-template control (NTC) reactions to detect contamination.

2. PCR Primer Design

Effective primer design is critical for high-fidelity amplification:

- Design primers of 15–30 nucleotides with 40–60% GC content.
- Ensure Tm difference between primers is ≤5°C, excluding non-template sequences.
- Avoid three or more consecutive G/C nucleotides at the 3'-end to minimize mispriming.
- Prefer 3'-terminal G or C to enhance specificity.
- Avoid self-complementary or inter-primer complementary sequences to prevent hairpins or dimers.
- Verify primer specificity using tools like NCBI BLAST.
- For degenerate primers, ensure ≥3 conserved nucleotides at the 3'-end.
- Avoid dUTP, dITP, or primers containing these nucleotides, as they inhibit *Pfu* DNA polymerase.

3. PCR Reaction Mixture Components

- **Template DNA**: Optimize concentration to balance yield and specificity. Excessive template increases non-specific products; insufficient template reduces yield. Remove inhibitors (e.g., phenol, EDTA) via ethanol precipitation and 70% ethanol washes.
- **Primers**: Use 0.1–1.0 µM per primer. Higher concentrations (0.3–1 µM) may be needed for degenerate or long primers.
- **$MgSO_4$ Concentration**: Optimize within 1–4 mM (standard: 1.5 ± 0.5 mM with 0.2 mM dNTPs). Adjust for chelators like EDTA, which binds Mg^{2+} stoichiometrically.
- **dNTPs**: Maintain 0.2 mM per nucleotide. Higher concentrations require proportional $MgSO_4$ adjustments to account for binding. Ensure equimolar concentrations of dATP, dCTP, dGTP, and dTTP.

4. PCR Cycling Parameters

- **Initial Denaturation**: 1–3 min at 95°C for ≤50% GC templates; extend to 5–10 min for GC-rich or complex templates.
- **Annealing**: Set 3–5°C below primer Tm; optimize for specificity.
- **Extension**: Use 2 min/kb at 72°C for ≤2 kb amplicons; reduce to 68°C for longer products to preserve enzyme activity.

- **Cycle Number**: Use 25–35 cycles for most templates; up to 40 cycles for <10 template copies.
- **Final Extension**: Extend to 5–15 min to ensure complete blunt-end products.

5. Reaction Setup

UniAccu *Pfu* DNA Polymerase retains activity at room temperature, potentially causing non-specific amplification. Prepare reactions on ice and transfer promptly to a preheated thermal cycler to enhance specificity.

Precautions and Disclaimer

This product is designated for research and development purposes only and is not intended for therapeutic, diagnostic, household, or other non-research applications. Handle using standard laboratory protective equipment, including lab coats, disposable gloves, and safety goggles. When using radioactive nucleotides, adhere to institutional radiation safety protocols. Comprehensive safety data are available in the Material Safety Data Sheets (MSDSs) at www.atrmed.com or via email request to info@atrmed.com. To the maximum extent permitted by applicable law, ATR-MED Inc. disclaims liability for special, incidental, indirect, punitive, or consequential damages arising from the use of this product or associated documentation. Product use constitutes acceptance of ATR-MED's terms and conditions. All trademarks are owned by ATR-MED unless otherwise specified.

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