Uni *Taq* DNA Polymerase (Mg^{2+} Plus Buffer)

Product Information

Uni *Taq* DNA Polymerase (Mg^{2+} Plus Buffer)

Kit Contents

Components	ATR-P501-2 (500 Units)
Uni *Taq* DNA Polymerase (5 U/µL)	100 µL
10X Uni *Taq* Reaction Buffer (with $MgCl_2$)	1 mL

Product Description

Uni *Taq* DNA Polymerase is a highly purified, recombinant enzyme derived through genetic engineering, optimized for robust 5'→3' DNA synthesis. This enzyme lacks detectable 3'→5' exonuclease (proofreading) activity but exhibits 5'→3' exonuclease activity, enabling efficient strand displacement during amplification. Additionally, Uni *Taq* DNA Polymerase demonstrates deoxynucleotidyl transferase activity, resulting in the addition of a single adenine nucleotide at the 3'-end of amplified products (A-tailing), which facilitates seamless ligation into TA cloning vectors. The enzyme is optimized for standard polymerase chain reaction (PCR) amplification of DNA fragments up to 3 kb, delivering high specificity and yield across diverse template-primer systems. Uni *Taq* DNA Polymerase surpasses the performance of conventional *Taq* DNA polymerases from other suppliers, as validated through rigorous comparative testing. Each production lot undergoes comprehensive quality control assessments, including stringent assays for PCR specificity, reproducibility, and fidelity. These tests confirm the enzyme's suitability for high-fidelity amplification of DNA templates, including plasmid, genomic, and cDNA, making it ideal for routine and high-throughput PCR applications.

Applications

- Routine PCR amplification of DNA fragments
- High-throughput PCR workflows
- TA cloning of PCR products
- DNA labeling and sequencing
- Reverse transcription PCR (RT-PCR) for cDNA amplification

Highlights

- High-yield and specific amplification across diverse primer-template systems
- Generates PCR products with 3'-dA overhangs, optimized for TA cloning
- Robust performance for amplicons up to 3 kb
- Exceptional lot-to-lot consistency and reproducibility

Source

Recombinantly expressed and purified from an Escherichia coli strain harboring the cloned 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 74°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, 50% (v/v) glycerol.

10X Uni *Taq* Reaction Buffer Composition

100 mM Tris-HCl (pH 8.8 at 25°C), 500 mM KCl, 15 mM $MgCl_2$, 1% (v/v) Triton X-100.

Storage

Store all kit components at -20°C.

Shipping

The kit is shipped on gel ice packs.

Protocol

To ensure optimal performance and minimize pipetting errors, prepare a PCR master mix containing all components except Uni *Taq* 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 Uni *Taq* Reaction Buffer (with $MgCl_2$)	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
Uni *Taq* DNA Polymerase (5 U/µL)	0.5-1 µL	2.5–5 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	1 min/kb	
Final Extension [d]	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 1 min/kb at 72°C for amplicons ≤2 kb; for >2 kb, reduce to 68°C to prevent enzyme inactivation.

[d] Extend to 30 min for TA cloning to optimize 3'-dA tailing.

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 to detectable levels:

- 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 specific 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.
- Use primer design software to incorporate restriction enzyme sites if needed.

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.
- $MgCl_2$ Concentration: Optimize within 1–4 mM (standard: 2.0 ± 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 $MgCl_2$ adjustments to account for binding.

4. PCR Cycling Parameters

- Initial Denaturation: 1–3 min at 95°C for ≤50% GC templates; extend to 3–10 min for GC-rich or complex templates.
- Annealing: Set 3–5°C below primer Tm; optimize for specificity.

- Extension: Use 1 min/kb at 72°C for ≤2 kb amplicons; reduce to 68°C for longer products.
- Cycle Number: Use 25–35 cycles for most templates; up to 40 cycles for <10 template copies.
- Final Extension: Extend to 15–30 min for TA cloning to ensure complete A-tailing.

5. Reaction Setup

Uni *Taq* DNA Polymerase retains activity at room temperature, potentially leading to 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.

Limited Product Warranty

ATR-MED® warrants that, at the time of quality release or retest, this product conforms to the specifications herein, pursuant to the General Terms and Conditions of Sale at www.atrmed.com/terms-and-conditions.html. For warranty inquiries, contact support at www.atrmed.com/support. Users must independently verify product suitability for their applications. Additional terms may be included on invoices or packing slips.

Trademarks

ATR-MED® is a registered trademark of Acell Teb Rad.