

Product Information

UniQ Red PCR Master Mix (2X)

Catalogue Number	Size
ATR-P509-1	1 mL (80 x 25 µL reactions)
ATR-P509-2	5 x 1 mL (400 x 25 µL reactions)

Product Description

ATR-MED® UniQ Red PCR Master Mix (2X) is a ready-to-use, optimized reagent containing recombinant *Taq* DNA polymerase, deoxynucleotide triphosphates (dNTPs), magnesium chloride (MgCl₂), and a proprietary buffer system designed to minimize optimization requirements. The master mix requires only the addition of primers, template DNA, and nuclease-free water to perform robust polymerase chain reaction (PCR) amplification, reducing pipetting steps and enhancing throughput and reproducibility. Benchmarked against leading commercial *Taq* master mixes, ATR-MED® UniQ Red PCR Master Mix (2X) demonstrates superior specificity and yield across a wide range of PCR applications. It supports amplification of DNA fragments up to 3 kb from diverse templates, including purified DNA, bacterial colonies, and cDNA, making it ideal for routine molecular biology workflows. Each production lot undergoes stringent quality control, including assays for PCR specificity, sensitivity, and reproducibility, ensuring consistent performance. The master mix incorporates protective agents to maintain enzymatic activity through up to 15 freeze-thaw cycles and allows storage at 2–8°C for up to 2 months, enabling convenient aliquot storage at 4°C for frequent use without thawing. An inert red tracer dye, which co-migrates with approximately a 125 bp fragment in a 1% agarose gel, facilitates direct loading of PCR products for electrophoresis without affecting amplification efficiency or downstream applications, such as nested PCR or TA cloning.

Applications

- High-throughput PCR amplification
- Routine PCR with high reproducibility
- Generation of PCR products for TA cloning
- Reverse transcription PCR (RT-PCR)
- Amplification from genomic DNA, cDNA, and plasmid templates

Highlights

- **High Specificity:** Ensures precise amplification across diverse primer-template systems.

- **Stability:** Retains full activity after 15 freeze-thaw cycles and up to 2 months at 4°C.
- **Convenience:** Includes red tracer dye for direct gel loading; compatible with nested PCR and TA cloning.
- **Robust Performance:** Optimized for amplicons up to 3 kb with minimal optimization.
- **Lot-to-Lot Consistency:** Rigorous quality control ensures reproducible results.

Source

Recombinant *Taq* DNA polymerase expressed and purified from *Escherichia coli* harboring the cloned *Thermus aquaticus* polymerase gene.

Buffer Composition

UniQ Red PCR Master Mix (2X): Proprietary formulation containing *Taq* DNA polymerase, 0.4 mM each dNTP (dATP, dCTP, dGTP, dTTP), 4 mM MgCl₂, reaction buffer, stabilizers, and inert red tracer dye.

Storage

Store at -20°C in a tightly closed container to maintain stability. For frequent use, an aliquot may be stored at 2–8°C for up to 2 months without loss of activity.

Shipping

Shipped on gel ice packs at ≤0°C to ensure stability. Transfer immediately to a -20°C freezer upon receipt.

Protocols

ATR-MED® UniQ Red PCR Master Mix (2X) is used at a 1X final concentration in a typical 25 µL reaction volume, scalable to 50 µL if needed.

1. Gently vortex and briefly centrifuge the master mix after thawing.
2. In a thin-walled PCR tube on ice, prepare the following reaction mixture for a 25 µL reaction:

Component	Amount
PCR Master Mix (2X)	12.5 µL
Forward primer	0.1–1.0 µM (final)
Reverse primer	0.1–1.0 µM (final)
Template DNA*	10 pg - 1 µg
Nuclease-Free Water	to 50 µL

*Recommended template DNA concentrations:

Template DNA	Concentration
Animal & Plant Genomic DNA	0.1 - 1 µg
<i>E. coli</i> 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

- Gently vortex the reaction mix and briefly centrifuge.
- If using a thermal cycler without a heated lid, overlay with 25 µL of mineral oil.
- 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	T _m -5	30 sec	
Extension ^c	72	1 min/kb	
Final Extension ^d	72	5-15 min	1

^a Extend to 5–10 min for complex templates (e.g., high GC content or secondary structures).

^b Set annealing temperature 3–5°C below the primer T_m; optimize in 1–2°C increments for complex templates.

^c Use 1 min for amplicons ≤1 kb; extend by 1 min/kb for longer products. For amplicons >2 kb, consider lowering to 68°C to preserve enzyme activity.

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

- Load 3–5 µL of PCR product directly onto an agarose gel. The red tracer dye co-migrates with ~125 bp in a 1% agarose gel. For enhanced tracking, run a common tracking dye in an unused lane if needed.

Important Notes

1. Contamination Prevention in PCR Setup

To mitigate contamination risks in PCR, which can amplify trace contaminants to detectable levels:

- Perform DNA preparation, PCR setup, amplification, and analysis in physically separated areas.

- Use a laminar flow cabinet with UV sterilization for PCR mixture preparation.
- Wear fresh gloves during DNA purification and reaction setup.
- Dedicate reagent containers and pipettes exclusively for PCR.
- Use positive displacement pipettes or aerosol-filtered tips.
- Employ PCR-certified reagents, including high-purity nuclease-free water.
- Include "no template control" (NTC) reactions to monitor contamination.

2. PCR Primer Design

Optimize primer design to ensure specificity and efficiency:

- Design primers of 15–30 nucleotides in length.
- Ensure primer T_m values differ by ≤5°C, excluding non-template sequences.
- Maintain 40–60% GC content with even distribution.
- Avoid >3 consecutive G or C nucleotides at the 3'-end to prevent non-specific priming.
- Prefer G or C at the 3'-end for stability.
- Minimize self-complementarity and inter-primer complementarity to avoid hairpins and 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:** Use optimal concentrations to balance yield and specificity. Remove inhibitors (e.g., phenol, EDTA, proteinase K) via ethanol precipitation and 70% ethanol washes.
- Primers:** Use 0.1–1 µM final concentration; 0.3–1 µM for degenerate or long primers to minimize mispriming.
- MgCl₂ Concentration:** The master mix contains 2 mM MgCl₂ (1X), optimized for 0.2 mM dNTPs. Adjust if chelators (e.g., EDTA) are present, as each EDTA molecule binds one Mg²⁺ ion.
- dNTPs:** The master mix provides 0.2 mM each dNTP. Ensure equal concentrations of dATP, dCTP, dGTP, and dTTP.

4. PCR Cycling Parameters

- **Initial Denaturation and Enzyme Activation:** Denature at 95°C for 1–3 min ($\leq 50\%$ GC content) or 5–10 min (complex/GC-rich templates) to ensure complete template unfolding.
- **Denaturation:** Use 95°C for 30 sec per cycle; extend to 3–4 min for GC-rich templates if needed.
- **Annealing:** Set 3–5°C below primer T_m ; optimize in 1–2°C increments if non-specific products occur.
- **Extension:** Use 72°C for 1 min/kb (≤ 1 kb amplicons); extend by 1 min/kb for longer products. For > 2 kb, lower to 68°C to preserve enzyme activity.
- **Cycle Number:** Use 25–35 cycles for > 10 template copies; 40 cycles for < 10 copies.
- **Final Extension:** Extend at 72°C for 5–15 min; use 30 min for TA cloning to enhance 3'-dA tailing.
- **Note:** Prepare reactions on ice to minimize non-specific amplification due to *Taq* polymerase activity at room temperature, then transfer to the thermal cycler.

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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