SYBR Green qPCR Master Mix, Low ROX (2X)

Product Information

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Catalogue Number	Size
ATR-P502-2	1 mL (100 x 20 µL reactions)

Product Description

ATR-MED® SYBR Green qPCR Master Mix, Low ROX (2X) is a ready-to-use reagent optimized for quantitative real-time polymerase chain reaction (qPCR) using SYBR Green I dye for fluorescence-based detection. The master mix contains a chemically modified, hot-start-activated UniTaq DNA polymerase, high-purity deoxynucleotide triphosphates (dNTPs), magnesium chloride ($MgCl_2$), a proprietary buffer system, SYBR Green I dye, low ROX reference dye, and stabilizers. This formulation minimizes non-specific amplification and primer-dimer formation, ensuring high sensitivity, specificity, and reproducibility, particularly for low-abundance templates. The hot-start UniTaq DNA polymerase exhibits 5'→3' polymerase and exonuclease activities but lacks 3'→5' exonuclease (proofreading) activity, requiring activation at 95°C for 10–15 minutes prior to cycling. The master mix includes all necessary components for qPCR, except template DNA and primers, streamlining setup and reducing pipetting errors. The low ROX reference dye corrects fluorescence signal variations across wells, making the master mix compatible with qPCR instruments requiring minimal ROX levels, including Applied Biosystems® 7500, 7500 Fast, ViiA™ 7, QuantStudio™ instruments, Agilent Mx3000P™, Mx3005P™, Mx4000™, and AriaMx. Benchmarked against leading SYBR Green master mixes, it delivers robust performance across a wide dynamic range, suitable for gene expression analysis, siRNA validation, genotyping, and pathogen detection. The master mix incorporates stabilizers to maintain activity through multiple freeze-thaw cycles and allows storage at 2–8°C for up to 1 month. Pre-assembled reactions remain stable for up to 72 hours at room temperature when protected from light, due to the photosensitivity of SYBR Green I and ROX dyes. The formulation ensures consistent cycle threshold (Ct) values, facilitating reliable quantitative results.

Applications

- Gene expression analysis
- siRNA validation
- Genotyping
- Pathogen detection

Highlights

- **High Specificity**: Hot-start UniTaq DNA polymerase and optimized buffer system suppress non-specific amplification and primer-dimer formation.
- **High Sensitivity**: Detects low-copy-number targets with high efficiency.
- **Stability**: Pre-assembled reactions stable for up to 72 hours at room temperature (in darkness); reagents stable at 4°C for up to 1 month.
- **Reproducibility**: Delivers consistent Ct values across a wide dynamic range, supported by a premixed 2X solution.
- **Instrument Compatibility**: Optimized with low ROX reference dye for specific Applied Biosystems® and Agilent platforms.

Source

Recombinant hot-start UniTaq DNA polymerase, chemically modified for activation, expressed and purified from an *Escherichia coli* strain harboring the cloned *Thermus aquaticus* polymerase gene.

Unit Definition

One unit (U) of UniTaq DNA polymerase 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.

Buffer Composition

SYBR Green qPCR Master Mix, Low ROX (2X): Proprietary formulation containing hot-start UniTaq DNA polymerase (0.05 U/µL in 1X), 0.4 mM each dNTP (dATP, dCTP, dGTP, dTTP), 4 mM $MgCl_2$, Tris-HCl, SYBR Green I dye, low ROX reference dye, stabilizers, and nuclease-free water.

Storage

Store at -20°C in a tightly closed container to maintain stability. Stable for at least 12 months at -20°C. For frequent use, an aliquot may be stored at 2–8°C for up to 1 month. Minimize exposure to light due to the photosensitivity of SYBR Green I and ROX dyes. Avoid repeated freeze-thaw cycles.

Shipping

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

Protocol

ATR-MED® SYBR Green qPCR Master Mix, Low ROX (2X) is used at a 1X final concentration in a typical 20 µL reaction volume. Smaller

volumes (<10 µL) are not recommended due to reduced fluorescence signal intensity.

1. Thaw the master mix on ice, gently mix without vortexing to avoid bubble formation, and briefly centrifuge to collect contents. Always include a no-template control (NTC).
2. In a thin-walled PCR tube or qPCR plate, prepare the following reaction mixture for a 20 µL reaction:

Component	Volume	Final Concentration
SYBR Green qPCR Master Mix, Low ROX (2X)	10 µL	1X
Forward primer (10 µM)*	0.5 µL (0.25 – 2 µL)	0.2 µM (0.1 – 0.8 µM)
Reverse primer (10 µM)*	0.5 µL (0.25 – 2 µL)	0.2 µM (0.1 – 0.8 µM)
Template DNA (or cDNA)**	Variable	1 to 100 ng
Nuclease-Free Water	to 20 µL	-

*Primer optimization: Conduct a titration (0.1–0.8 µM final concentration) to balance specificity and efficiency. Typically, 0.25 µM per primer is optimal. Increase concentration for low-abundance targets; decrease if non-specific products or primer-dimers occur.

**Template quantity: Perform gradient dilution to determine optimal input, not exceeding 10% of reaction volume (e.g., 1–2 µL of a 10-fold diluted cDNA from 1 µg RNA in a 10 µL reverse transcription reaction). Use 1 µL for standard templates to minimize inhibitor carryover; up to 5 µL for low-copy targets.

3. Gently mix to avoid bubble formation and briefly centrifuge.
4. Perform qPCR using the recommended thermal cycling conditions. The three-step protocol is preferred for optimal specificity, but a two-step protocol may be used for faster cycling:

Three-Step qPCR Program

Step		Temperature (°C)	Time	Number of Cycles
Initial Denaturation *		95	10-15 min	1
PCR	**Denaturation**	95	15 sec	40
	Annealing **	50-60	30 sec	
	Extension ***	72	30 sec	
Melt Curve		95	15 sec	1
		60	60 sec	
		95	15 sec	

Two-Step qPCR Program

Step		Temperature (°C)	Time	Number of cycles
Initial denaturation *		95	10 min	1
PCR	**Denaturation**	95	15 sec	40
	Annealing **	60	30-60 sec	
Melt Curve		95	15 sec	1
		60	60 sec	
		95	15 sec	

* Extend to 15 min for complex templates (e.g., high GC content or secondary structures).

** Set annealing temperature 3–5°C below primer Tm; optimize in 1–2°C increments for specificity.

*** For amplicons >300 bp, extend by 1 min/kb, as UniTaq polymerase extends at ~1000 bp/min.

5. **Analyze results:**

Amplification Curve: Optimal Ct values range from 15–35, with 20–28 preferred. Adjust template dilution if Ct is too low (<15) or too high (>35).

Melting Curve: A single peak indicates specific amplification. Multiple peaks suggest primer-dimers or non-specific products, requiring primer redesign or optimization of annealing temperature.

Important Notes

1. Contamination Prevention in qPCR Setup

To mitigate contamination risks, which can compromise qPCR sensitivity:

- Perform template preparation, reaction setup, amplification, and analysis in physically separated areas.
- Use a laminar flow cabinet with UV sterilization for reaction setup.
- Wear fresh gloves during template preparation and reaction setup.
- Dedicate reagent containers and pipettes exclusively for qPCR.
- Use positive displacement pipettes or aerosol-filtered tips.
- Employ qPCR-certified reagents, including high-purity nuclease-free water.
- Include no-template control (NTC) reactions to monitor contamination.

2. qPCR Primer Design

Optimize primer design for specificity and efficiency:

- Design primers of 18–24 nucleotides with 40–60% GC content.
- Ensure primer Tm values differ by ≤5°C, calculated excluding non-template sequences.
- 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 primer-dimers.
- Verify specificity using tools like NCBI BLAST.
- Design amplicons of 100–200 bp for optimal qPCR efficiency.
- For gene expression, design primers spanning exon-exon junctions or introns to avoid genomic DNA amplification.

3. qPCR Reaction Mixture Components

- **Template DNA/cDNA**: Use 1–100 ng, optimized via gradient dilution. Limit to ≤10% reaction volume to minimize inhibitors. For low-abundance targets, use up to 5 µL cDNA.
- **Primers**: Use 0.1–0.8 µM final concentration; 0.25 µM is typically optimal. Adjust for low-abundance targets or to reduce primer-dimers.
- **$MgCl_2$ Concentration**: The master mix contains 2 mM $MgCl_2$ (1X), optimized for 0.2 mM dNTPs. Adjust if chelators (e.g., EDTA) are present.
- **dNTPs**: The master mix provides 0.2 mM each dNTP, balanced for optimal performance.

4. qPCR Cycling Parameters

- **Initial Denaturation and Enzyme Activation**: Use 95°C for 10–15 min to activate hot-start polymerase and denature complex templates.
- **Denaturation**: Use 95°C for 15 sec per cycle.
- **Annealing**: Set 3–5°C below primer Tm; optimize in 1–2°C increments if non-specific amplification occurs.
- **Extension**: Use 72°C for 30 sec for amplicons ≤300 bp; extend by 1 min/kb for longer amplicons.
- **Cycle Number**: Use 40 cycles for optimal sensitivity.
- **Melt Curve**: Perform to confirm product specificity; a single peak indicates specific amplification.
- **Note**: Prepare reactions on ice to prevent non-specific amplification. Minimize light exposure during setup to protect SYBR Green I and ROX dyes.

Troubleshooting

Problem	Potential Cause(s)	Solution(s)
Amplification in NTC	Contaminated reagents/water	Use fresh reagents and nuclease-free water; work on a clean bench.
	Primer-dimers	Normal after 35 cycles; confirm with melting curve analysis. Redesign primers if dimers persist.
Ct Too High/Low	Low amplification efficiency	Optimize using three-step protocol or redesign primers.
	Low template concentration	Increase template input or reduce dilution.
	Template degradation	Prepare fresh template.
	Amplicon too long	Design amplicons of 100–200 bp.
	PCR inhibitors	Dilute or re-purify template to remove inhibitors.
Abnormal Amplification Curves	Weak signal	Increase template concentration to improve signal strength.
	Fractured/descending curve	High template concentration; reduce baseline endpoint (Ct minus 4) and re-analyze.
	Sudden drop in curve	Bubbles in reaction; centrifuge to remove bubbles before cycling.
No Amplification Curve	Insufficient cycles	Set to 40 cycles.
	No signal collection	Ensure signal collection at extension (three-step) or annealing/extension (two-step).
	Primer degradation	Verify primer integrity via PAGE.
	Low template concentration	Reduce dilution or use undiluted template for low-expression genes.
	Template degradation	Prepare fresh template.
Multiple Melting Curve Peaks	Poor primer design	Redesign primers to avoid dimers (peaks at ~75°C).
	High primer concentration	Reduce primer concentration.
	Low template concentration	Increase template input.
	Genomic DNA contamination	Design intron-spanning primers.
Poor Reproducibility	Sampling error	Increase reaction volume or template input.
	Low template concentration	Increase template amount.
	Instrument issues	Calibrate the instrument to ensure uniform well temperatures.

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. Minimize light exposure to protect SYBR Green I and ROX dyes. 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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