Product Information

SYBR Green qPCR Master Mix, No ROX (2X)

Catalogue Number	Size
ATR-520-3	1 mL (100 x 20 µl reactions)

Product Description

SYBR Green qPCR Master Mix, No ROX (2X), employs a specialized UniTaq DNA polymerase, activated through a hot-start technique, and a finely tuned buffer system for conducting quantitative PCR (qPCR) using SYBR Green I dye. The chemically modified Hot-Start UniTaq DNA polymerase, accompanied by a proprietary reaction buffer, facilitates robust qPCR performance, particularly with low-template samples, ensuring heightened sensitivity, specificity, and reliability while mitigating nonspecific amplification during setup. This polymerase exhibits 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading). Activation of the enzyme at 95°C for 10-15 minutes is imperative before utilization. The SYBR Green qPCR Master Mix, No ROX (2X), comprises all requisite components for qPCR, excluding templates and primers.

SYBR Green qPCR Master Mix, No ROX (2X) is especially suited for the following instruments: Bio-Rad CFX96 Touch™, CFX384 Touch™, CFX Connect™, DNA Engine Opticon® 2, Chromo4™, iCycler iQ™ and My iQ™, Roche LightCycler® 480, LightCycler® 1536, LightCycler® Nano, LightCycler® 96 and QuantStudio™ instruments, Thermo Scientific™ PikoReal™, Cepheid SmartCycler®, Bio Molecular Systems Mic qPCR cycler, Qiagen Rotor Gene Q, Rotor Gene 6000, MyGo Mini and MyGo Pro

Applications

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

Highlights

- **Specificity**: The Hot-Start UniTaq DNA polymerase combined with the optimized buffer effectively suppresses non-specific amplification and primer dimer formation.
- **Sensitivity**: Enables detection of targets with low copy numbers.
- **Stability**: Pre-assembled reactions exhibit high stability when incubated in darkness at room temperature for up to 72 hours.
- **Reproducibility and convenience**: Offers highly reproducible cycle threshold (Ct) values across a wide dynamic range, facilitated by the premixed all-in-one 2X solution.

Storage

Stored at -20°C. SYBR Green qPCR Master Mix, No ROX (2X) is stable for a minimum of 12 months. The reagents can be stored at 4 °C for up to 1 month. Avoid repeated freeze-thawing.
The ROX and SYBR Green dyes are light-sensitive; exposure should be minimized.

Shipping

The kit is shipped with ice gel.

Protocols

Thaw **SYBR Green qPCR Master Mix, No ROX (2X)**, on ice, gently mix, and centrifuge briefly to recover maximum volume without vortexing to prevent introduction of bubbles that may disrupt fluorescence. Always incorporate a no template control (NTC).

Utilize the SYBR Green qPCR Master Mix, No ROX (2X), at a 1X concentration in a total reaction volume of 20 µL with template and primers.

Scale all components proportionally for smaller reaction volumes, with volumes < 10 µL discouraged due to diminished signal intensity.

1. Add the following components for each 20 µL reaction in a thin-walled PCR tube:

Component	Volume	Final Concentration
SYBR Green qPCR Master Mix, No 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
Water, nuclease-free	to 20 µL	-
Total volume	20 µL	-

*For primer optimization, conduct a titration ranging from 0.2 µM to 1 µM final concentration, adjusting volume and concentration as needed. Typically, maintain upstream and downstream primer concentrations at 0.5 µM to ensure optimal outcomes. If amplification efficiency is inadequate, elevate primer concentration; conversely, decrease concentration if specificity is compromised. For low-abundance genes, consider employing a reaction volume exceeding 20 µL.

** The quantity of template added depends on target gene copies, with gradient dilution preferred for determining optimal addition, generally not surpassing 10% of the total system. For instance, a 10-fold dilution of 1 µg RNA reverse transcription product (10 µL reverse system) is recommended as a template, with 1 µL typically added to a 20 µL qPCR system. Adjust template quantity accordingly for low-abundance genes. For standard templates, limit addition to 1 µL to mitigate PCR inhibitor carryover; this may be increased to 5 µL for low-copy templates.

2. Gently mix to avoid bubble formation and centrifuge briefly.

3. SYBR Green qPCR Master Mix, No ROX (2X), is suitable for both two-step and three-step procedures, though the latter is preferable. In cases of poor reaction performance, adjustments can be made: prolong thermal start time, reduce annealing temperature, or increase extension time for enhanced amplification efficiency; adjust annealing temperature accordingly to improve amplification specificity.

Three-Step PCR Program

Step		Temperature (°C)	Time	Number of cycles
Initial denaturation [a]		95	10 min	1
PCR	**Denaturation**	95	15 sec	40
	Annealing [b]	50-60	30 sec	
	Extension [c]	72	30 sec	
Melt Curve		95	15 sec	1
		60	60 sec	
		95	15 sec	

Two-Step PCR Program

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

[a] The duration of initial denaturation is a crucial parameter in amplification reactions and can be tailored based on the complexity of the template structure. For templates with intricate structures, extending the initial denaturation time up to 15 minutes enhances its efficacy.
[b] The annealing temperature depends on the primer sequence.
[c] The extension time is influenced by the length of the amplicon. For amplicons exceeding 300 base pairs, the adaptation of amplification time is warranted, as Hot-Start UniTaq DNA polymerase extends at approximately 1000 base pairs per minute.

Result analysis

Amplification curve: Generally, the ranges of CT values are 15 to 35, while 20-28 are the best. If the CT value is too low, increase the dilution ratio of the template. If the CT value is too high, raise the concentration of templates or primers, and even adjust the qPCR program.

Melting curve: Usually only when the melting curve is single peak, the quantitative result can be qualified. If there are multiple peaks in the dissolution curve, it is necessary to optimize the conditions, such as redesign primers.

Precautions and Disclaimer

This product is intended for research and development (R&D) purposes only and is not suitable for use in drugs, diagnostic procedures, households, or other applications. When handling the product, always wear appropriate laboratory attire, including a lab coat, disposable gloves, and protective eyewear. When working with radioactive tracers, adhere to standard safety protocols for handling radioactive materials. For detailed safety information, refer to the relevant material safety data sheets (MSDSs), which are available online as PDF files or upon request via email (info@atrmed.com). To the fullest extent permitted by law, ATR-MED Inc. disclaims liability for any special, incidental, indirect, punitive, multiple, or consequential damages arising from or related to the use of this document, including any associated products. By using this product, you acknowledge and agree to all terms and conditions outlined by ATR-MED. All trademarks mentioned herein are the property of ATR-MED unless otherwise specified.

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Troubleshooting

Problem	Potential Cause(s)	Solution(s)
Amplification in the negative control	Reagent or water used is contaminated	Use new reagents and de-ionized water when operating in a clean laboratory bench
	Primer dimers	It is normal to produce amplification curves in the negative control after 35 cycles. Please analysis the results according to their melting curves.
Ct is higher or lower than the normal values	Low amplification efficiency	Optimize the reaction system, try three-step method or redesign primers
	Low template concentration	Increase the concentration of template
	Template degradation	Prepare fresh template
	Too long amplification fragments	The length of amplification fragments should be 100-200 bp
	PCR inhibitors exist in reaction system	Try to dilute or re-prepare the template, because inhibitors were usually added with the template.
Abnormal amplification curves	Abnormal shape of the amplification curve	When the signal is weak, the system calibration may lead to this result, which can be corrected by increasing the template concentration.
	Fractured or descending shape of the amplification curve	When the template concentration is too high, the baseline endpoint value is higher than the CT value. Decrease the baseline endpoint value (Ct value minus 4) and re-analysis the data.
	Suddenly falling shape of the amplification curve	The bubbles in the reaction system will burst suddenly when the temperature rises. The equipment will detect a sudden drop in fluorescence value. Centrifuge and check whether there are bubbles to avoid this problem.
Without a amplification curve	Insufficient cycle number	The cycle number is usually set to be 40
	No signal collection procedure during cycling	In two-step program, signal collection is usually positioned at annealing and extension stage; for three-step program, signal collection should be positioned at 72°C extension stage
	The primer degradation	After long-term storage, the integrity of primers should be confirmed by PAGE gel
	The template concentration is too low	Decrease the dilution ratio (For target genes with unknown expression, their template was used without dilution for the first time)
	The template degradation	Prepare fresh template
Heterozygous peak of melting curve	Unreasonable primer design	The undesired peaks of primer dimers often occur at about 75°C. If the peak is significant, the primers need to be redesigned.
	The primer concentration is too high	Decrease primer concentration properly
	The template concentration is too low	Increase template concentration
	The contamination of genome DNA	Design primers by transcending introns
Poor stability of duplicated wells	Sampling error	Increase the reaction system; Increase the dilution ratio and sampling volume of templates
	The template concentration is too low	Increase the sample size
	Instrument problems	The temperature of each hole varies, so it is necessary to calibrate the instrument before use.