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

Instructions for Use of Product ATR-520-1.

Quick Protocol

SYBR Green qPCR Master Mix, High ROX (2X), employs a 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.

Storage

Stored at -20°C.

Protocols

Thaw **SYBR Green qPCR Master Mix, High ROX (2X)**, on ice, gently mix, and centrifuge briefly to recover maximum volume without vortexing to prevent the formation of bubbles that may disrupt fluorescence. Always incorporate a no template control (NTC). Utilize the **Green qPCR Master Mix, High ROX (2X)**, at a 1X concentration in a total reaction volume of 20 µL with template and primers.

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, High 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, High 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.

Additional protocol information is available in Product Information #ATR-P520-1, available online at: www.atrmed.com