

Product Information

SYBR[®] Green ReadyMix[™] for High Throughput Quantitative PCR

Product Code S 9194

Storage Temperature -20 °C

TECHNICAL BULLETIN

Product Description

SYBR Green ReadyMix for High Throughput Quantitative PCR combines the performance enhancements of JumpStart[™] Taq and SYBR Green I in an easy-to-use ReadyMix solution that incorporates ROX dye for ABI and other real time instrument applications. The ReadyMix includes a detection fluor, internal standard and reagents for PCR making it the ideal solution for performing high-throughput quantitative PCR. The ReadyMix contains SYBR Green I, JumpStart Taq DNA polymerase, 99% pure deoxynucleotides, and reaction buffer. It is provided in a 2x concentrate. Simply add an equal volume of the ReadyMix to a 2x mixture of DNA template and primers.

JumpStart Taq antibody inactivates the DNA polymerase at room temperature, thereby preventing non-specific product formation. When the temperature is raised above 70 °C in the first denaturation step of the cycling process, the complex dissociates and the polymerase becomes fully active. Antibody-mediated hot start requires no special preparations or protocol changes.

Sigma's Reference Dye for Quantitative PCR is incorporated in the mix to normalize reaction data for real-time detection. Maximum excitation and emission of the dye is 586 nm and 605 nm, respectively. Instrument settings for ROX reference dye are satisfactory for the measurement of Reference Dye for Quantitative PCR.

Features and Benefits

- SYBR Green ReadyMix for High Throughput Quantitative PCR is the perfect ReadyMix for high throughput, quantitative PCR applications.
- SYBR Green I is ideal for quantifying any DNA sequence.¹ The dye binds to double-stranded DNA and detection is monitored by measuring the increase in fluorescence throughout cycling.

- The hot start mechanism, using JumpStart Taq antibody, prevents non-specific product formation and allows assembled PCR reactions to be placed at room temperature up to 2 hours without compromising performance.
- Internal Reference Dye is provided for reaction normalization. Maximum excitation and emission of the dye is 586 nm and 605 nm, respectively.
- When performing large numbers of PCR reactions, the SYBR Green JumpStart Taq ReadyMix can save a significant amount of preparation time, reduce the risk of contamination from multiple pipetting steps, and provide consistent batch-to-batch and reaction-to-reaction performance.

Reagents

Sufficient for 400 or 2000 PCR reactions (50 µl reaction volume)

SYBR Green JumpStart Taq ReadyMix, Product Code S 9939, containing 20 mM Tris-HCl, pH 8.3, 25 °C, 100 mM KCl, 7 mM MgCl₂, 0.4 mM each dNTP (dATP, dCTP, dGTP, TTP), stabilizers, 0.05 unit/µl Taq DNA Polymerase, JumpStart Taq antibody, 2x internal reference dye and SYBR Green I.

Reagents and Equipment Required But Not Provided (Product Codes have been given where appropriate)

- PCR Reagent Water, Product Code W 1754
- Primers
- DNA template
- Dedicated pipettes
- PCR pipette tips
- 0.2 ml or 0.5 ml thin-walled PCR microcentrifuge tubes or plates for specific thermal cycler
- Real time instrument for quantitative PCR

Precautions and Disclaimer

SYBR Green ReadyMix for High Throughput Quantitative PCR is for R & D laboratory use only. Not for drug, household or other uses.

Storage/Stability

SYBR Green ReadyMix for High Throughput Quantitative PCR can be stored at 2-8 °C for up to 3 months or at -20°C for up to one year. There was no detectable loss of performance after 10 freeze-thaw cycles.

Procedure

Note: Because SYBR Green I binds to all double-stranded DNA, it is important to test primers and cycling conditions to insure that the PCR product is a single band. It is best to insure PCR specificity by checking the reaction on a normal (non-quantitative) thermocycler and analyzing the result using agarose gel separation.²

Optimal concentrations of template DNA, MgCl₂, KCl, and PCR adjuncts as well as pH are often target specific. Optimization may be needed for specific template and primers. Additional components (MgCl₂, dNTP, etc.) may be added to the template/primer mixture, although this is not required for most applications. The following procedure serves as a reference.

Note: The use of up to 5% (v/v) dimethyl sulfoxide (DMSO) will not disturb the enzyme-antibody complex, but will affect SYBR signal and background fluorescence. Other cosolvents, solutes (salts) and extremes in pH or other reaction conditions may reduce the affinity of the JumpStart *Taq* antibody for the *Taq* polymerase and thereby compromise its effectiveness.

1. Add the following reagents to the proper tube or plate for thermocycling.

Volume*	Component
25 µl	SYBR Green ReadyMix for High Throughput Quantitative PCR. Mix provides 3.5mM MgCl ₂ , final concentration.
w µl	Forward primer, 0.2 µM final concentration
x µl	Reverse primer, 0.2 µM final concentration
y µl	Template DNA
z µl	Water
50 µl	Total volume

* For 50 µl reaction, but component volumes may be scaled to give the desired final volume.

2. Mix gently by vortexing and briefly centrifuge to collect all components at the bottom of the tube.
3. Optimum cycling parameters vary with PCR composition and thermal cycler. It may be necessary to optimize the cycling parameters to achieve maximum product yield and/or quality.

Typical cycling parameters for 100 bp – 600 bp fragments:

Initial denaturation	94 °C	2 min.*
40 cycles:		
Denaturation	94 °C	15 sec.
Annealing/Extension	60 °C	1 min.**

* Initial denaturation of greater than two minutes is not recommended, and is unnecessary (see Troubleshooting Guide, Initial Denaturation).

** Detection is usually accomplished at this step.

References

1. Morrison, T. B., *et al.*, Quantification of Low-Copy Transcripts by Continuous SYBR[®] Green I Monitoring during Amplification. *BioTechniques*, **24**, 954-962 (1998).
2. Sambrook, J., and Russell, D. W., *Molecular Cloning: A Laboratory Manual*, Third Edition, (Cold Spring Harbor Laboratory Press, New York, 2001). (Product Code M 8265)

† The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

Troubleshooting Guide

Symptom	Possible Cause	Solution
No PCR product (signal) is observed	A PCR primer is missing or degraded.	A positive control should always be run to insure components are functioning. A checklist is also recommended when assembling reactions.
	Too few cycles are performed.	Increase the number of cycles.
	The annealing temperature is too high.	Decrease the annealing temperature in 2-4 °C increments.
	The primers are not designed optimally.	Confirm the accuracy of the sequence information. If the primers are less than 27 nucleotides long, try to lengthen the primer to 27-33 nucleotides. If the primer has a GC content of less than 45%, try to redesign the primer with a GC content of 45-60%.
	There is not enough template.	After increasing the number of cycles has shown no success, repeat the reaction with a 10-fold higher concentration of the template.
	The template is of poor quality.	Evaluate the template integrity by agarose gel electrophoresis. It may be necessary to repurify template using methods that minimize shearing and nicking.
	PCR product is too long.	For optimal results, qPCR products should be 100-500 bp.
	Target template is difficult.	In most cases, inherently difficult targets are due to unusually high GC content and/or secondary structure. Betaine has been reported to help amplification of high GC content templates at a concentration of 0.8-1.3 M. [Rees, W., <i>et al.</i> , <i>Biochemistry</i> , 32 , 137-144 (1993).]
Signal is independent of template dilution (multiple products or smeared products)	The annealing temperature is too low.	Increase the annealing temperature in increments of 2-3 °C.
	The primers are not designed optimally.	Confirm the accuracy of the sequence information. If the primers are less than 27 nucleotides long, try to lengthen the primers to 27-33 nucleotides. If the primer has a GC content of less than 45%, try to redesign the primers with a GC content of 45-60%.
	The template concentration is too high.	Reduce the concentration of the template in the PCR reaction.
	The primer concentration is too high.	Reduce the primer concentrations in a series of two-fold dilutions (i.e. 0.1 µM, 0.05 µM, 0.025 µM, and 0.0125 µM) and subject these trial reactions to PCR.
Large variability within samples and/or duplicates.	Reactions not well mixed.	Gently vortex and centrifuge reactions.
	Wells not tightly capped or covered.	Tightly cap or cover all wells, even the empty ones. Loose caps can compromise the seal of adjacent wells.
	Initial denaturation is too long.	Decrease initial denaturation to not exceed two minutes.
No PCR product (signal) is observed.	Wrong dye layer chosen.	Ensure the reporter being used is activated in the setup view of the Sequence Detection Software.
	Incorrect values on Y-axis	Change the values on the y-axis. By doubling clicking on ΔRn, the values of the y-axis can be changed.

Varying fluorescent intensity	Amplification curves reach a maximum and then decrease at high template amounts	Reduce the number of cycles used for baseline calculation. Baseline correction is overcompensating, and negating signal.
	Improper exposure time	Change the exposure time appropriately if using caps (25) or optical adhesive covers (10).

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