User Guide

LuminoCt® SYBR® Green qPCR ReadyMix™

L6544

Directions for Use

LuminoCt® SYBR® Green qPCR ReadyMix® combines the performance enhancements of our JumpStart™ Taq antibody for hot start[1] PCR with the convenience of an easy-to-use reaction mixture. This is the ideal solution for performing high-throughput, quantitative PCR methods using a SYBR® detection method.

This ready-to-use mixture of JumpStart™ Taq DNA polymerase, SYBR® Green I, 99% pure deoxynucleotides and reaction buffer is provided in a 2X concentrate for ease-of-use. Simply add an equal volume of the 2X mix to the DNA template, primers, and water. At room temperature, the JumpStart Taq antibody inactivates the Taq DNA polymerase. 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. This process is rapid (seconds) and therefore fast enough, so there is no requirement for long activation, special preparation or cycling changes.

LuminoCt® SYBR® Green qPCR ReadyMix™ works best with small amplicons (<200 bp) and are compatible with commercial primer sets, including TaqMan® assays.

Features

- Enables rapid, high-throughput 2-step qPCR with results in ~25 minutes
- Provides consistent, reproducible performance across reactions
- Minimizes prep time and contamination risk by reducing pipetting steps
- Compatible with common real-time PCR platforms and probe-based assays

Hot-start JumpStart™ *Taq* allows room-temperature setup and prevents non-specific amplification.

Applications

LuminoCt® SYBR® Green qPCR ReadyMix™ has been used for fast quantitative polymerase chain reaction (qPCR) and quantitative RT-PCR amplifications.[2,3,4,5,6] It has also been used in qPCR for measuring gene expression.[7]

Reagents Provided

- LuminoCt® SYBR® Green qPCR ReadyMix™, L5669, contains optimized concentrations of Tris-HCl, pH 8.3, KCl, dNTPs (dATP, dCTP, dGTP, TTP), stabilizers, $MgCl_2$, SYBR Green I and Jumpstart™ *Taq* DNA Polymerase. Provided as 100, 500 and 2000 reactions (25 µL mix in a 50 µL reaction volume).
- 100X ROX internal reference dye, R4526. Optional, for use with instruments, compatible with an internal reference dye (e.g. ABI and Stratagene® system).

Reagents Required

(Not included, see Product Ordering)

- Water, PCR Reagent, Catalog Number W1754
- Custom ordered primers/probes specific to gene target
- DNA template
- Dedicated pipettes with PCR pipette tips
- PCR tubes or plates
- Dedicated pipettes
- Sample containing template DNA to be amplified
- Quantitative (or real time) thermal cycler

Intended Use

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

SYBR Green I is photolabile, but when protected from light LuminoCt® SYBR® Green qPCR ReadyMix™ can be stored at –20 °C for up to a year and a half.

Directions for Use

Assemble Reaction Mix

For best reproducibility when performing multiple reactions, assemble a template/primer master mix by multiplying the volume needed times the number of reactions to be performed (plus 10% to account for pipetting error). Aliquot reaction mixture into PCR tubes.

Reagent	Final Concentration	Amount per 20 µL reaction	Amount per 50 µL reaction
LuminoCt® SYBR® Green qPCR ReadyMix™ (2x)	1X	10 µL	25 µL
$MgCl_2$	1.5–7 mM	Optional	Optional
Primers	0.1–1.0 µM	Variable	Variable
Template	Variable	Variable	Variable
Nuclease-free Water-free Water	–	To 20 µL total	To 50 µL total

Note: LuminoCt® SYBR® Green qPCR ReadyMix™ contains a magnesium ion-dependent enzyme. Optimal concentrations of template DNA, primers, and $MgCl_2$ as well as pH will be target-specific. For more information on ideal concentrations based on application, see Technical Guide.

Add Template

Recommended input template is 10 ng DNA; however, LuminoCt® SYBR® Green qPCR ReadyMix™ may amplify as little as a single copy of non-complex template or 10–100 copies of complex genomic template. For cDNA templates use a 1:10 reaction dilution for medium to highly expressed targets, or a 1:2 to 1:5 dilution for low expression targets.

Amplify

A suggested thermocycling protocol using LuminoCt® SYBR® Green qPCR ReadyMix™ is provided below:

Initial denaturation		94 °C	2 minutes
25–40 cycles	Denaturation	94 °C	15 seconds
	Annealing	60 °C or 5 °C below lowest primer T_m	30 seconds
	Extension	72 °C	1 minute/kb
Final extension		72 °C	1 minute
Hold		4 °C	∞

Amplification parameters will vary depending on primers, template, and instrument used. For tips on optimizing PCR conditions as well as a 2-step cycling protocol, please see the Technical Guide section.

Evaluate

The amplified DNA can be loaded directly onto an agarose gel after the PCR process. It is not necessary to add a separate loading buffer/tracking dye. Data can be processed via thermal cycler's associated program.

Technical Guide

Considerations for Primer Design

Thoughtful primer design is essential for PCR efficiency and specificity. For successful amplification consider the following:[8]

- Select an 18–30 nucleotide-long sequence with 40–60% G/C content and even distribution of all 4 bases.
- Avoid repetitive elements or self-complementary sequences >3 bp.
- Primer pairs should not differ in length by >3 bp and should not contain complementarity to one another.
- Maintain calculated primer T_m between 55–60 °C, permitting only 2–3 °C variation between primer pairs.
- Priming efficiency can be increased by including a terminal G at the 3' end; however, the number of Gs or Cs in the last 5 bases of the primer sequence should be no more than 3.
- Ensure each primer sequence is unique to the gene of interest and is absent in other genes in the gDNA sample or within the vector.

Optimization of PCR Conditions

PCR involves the cycling of denaturing, annealing, and extension steps for DNA synthesis by a polymerase enzyme. To obtain the best product yield and accuracy, each step must be optimized.

- The **denaturing** step (94–96 °C) activates the JumpStart™ *Taq* DNA Polymerase and separates double-stranded DNA strands, making it accessible to primers.[2] The duration of this step should be long enough to denature DNA but not so long that it compromises *Taq* DNA polymerase integrity. High salt conditions, GC-rich (>55%) templates, and gDNA templates may require longer denaturation times and/or higher temperatures. For maximum retention of JumpStart™ *Taq* activity during thermocycling, use 94 °C for denaturation.
- The **annealing** temperature can be calculated by subtracting 5 °C from the lowest reaction primer T_m. The annealing time should be long enough for the primer to anneal to the template but not too long for non-specific annealing to occur.[8]
- The optimal **extension** temperature for *Taq* DNA Polymerase is 72 °C; however, lower temperatures may be used for some reactions. Extension time depends on length and complexity of the target sequence. For complex templates, use 1 minute/kb, with 15 seconds added if the PCR product is >2 kb. Short or non-complex templates may be amplified with extension times of 30 seconds/kb.
- The **number of cycles** needed for amplification depends on the amount of template input, with higher amount of input requiring less cycling.[8] Generally, 25–30 cycles are sufficient to produce detectable product; however, low concentration templates may require up to 45 cycles.
- To maintain **enzyme fidelity**, or accuracy of nucleotide incorporation, limit the number of PCR cycles and use an equimolar concentration of each dNTP. Low magnesium concentration is also important to maintain enzyme fidelity.[9]

Handling gDNA Templates

To prevent genomic DNA (gDNA) shearing, add template last and mix gently using a wide pore pipet tip. DO NOT VORTEX!

Multiplex PCR

When performing multiplex PCR, competition between products for reagents may occur. Consider adjusting the following for optimization:[10]

- Proportion of primer pair concentration: if a target sequence produces a relatively "weaker" signal, the amount of primer used may be increased to compensate. For sequences with low copy numbers, or high-complexity, primer concentration can be used at 0.3–0.5 µM.
- Primer concentration can also be decreased for target sequences producing "stronger" signal to achieve balance. For high copy number or low-complexity sequences, primer concentration can be used at 0.04–0.4 µM.
- dNTP: Perform a stepwise increase of dNTP to a concentration ≤ 400 µM. Keep $MgCl_2$ concentration constant for this optimization.

  Note: The 2X master mix contains 400 µM dNTP.
- Supplement the reaction with additional $MgCl_2$ or PCR-enhancing additives.

PCR-Enhancing Additives

When optimizing PCR conditions for a new experiment, the following can be added to the reaction mix individually. After performing PCR amplification, samples with and without additive can be compared using agarose gel electrophoresis or other standard methods to look for improved product specificity and yield.

Additive	Purpose
BSA (10–100 µg/mL)	*Taq* DNA polymerase stabilization[11]
Formamide (1.25–10%)	Increases specificity in G/C rich regions[12]
DMSO (Up to 5%)	Accelerates strand renaturation[12] Nucleic acid thermal stability against depurination[12]
Glycerol (Up to 10%)	Increases thermal stability of the polymerase and lowers the temperature necessary for strand separation[13]
Ammonium sulfate (15–30 mM)	Affects the denaturing and annealing temperatures of the DNA[14]
Single strand binding protein (0.7–1.5 µg)	Inhibits formation of secondary structures, improving fidelity and *Taq* processivity[15]
Betaine (0.8–1.6 M)	Reduces base pair composition dependence of DNA melting[16]

Two-Step PCR Amplification

Application of a two-step PCR process is possible when the annealing and extension temperatures are similar.

Initial denaturation		94 °C	3 minutes
40 Cycles	Denaturation	94 °C	3 seconds
	Annealing/ extension	60 °C*	15–30 seconds
Hold		4 °C	∞

*Consult primer T_m regarding temperature selection. Extension time is target dependent, with larger targets requiring more than the recommended time.

Recommended $MgCl_2$ Concentrations by Application

Because JumpStart™ *Taq* DNA Polymerase is a magnesium ion-dependent enzyme, the optimal concentrations of template DNA, primers, and $MgCl_2$ will be target-dependent. The optimal $MgCl_2$ concentration is also dependent upon the intended application. See table below for recommended ranges of $MgCl_2$ to use in reactions containing JumpStart™ *Taq* DNA Polymerase.

Application	Recommended $MgCl_2$ Concentration Range
Endpoint PCR	1.5–3.5 mM
SYBR® green-based qPCR	3–5 mM
Probe-based qPCR	4–7 mM

Note: The supplied 2X JumpStart™ *Taq* ReadyMix™ contains 3 mM $MgCl_2$ for a working concentration of 1.5 mM.

Troubleshooting Guide

Problem	Suggestions
No or low product amplification	• Titrate $MgCl_2$ concentration in 0.5 mM increments using molecular biology grade $MgCl_2$. See Recommended $MgCl_2$ Concentrations by Application section in the Technical Guide for expected concentration ranges based on application. Each amplicon target must be optimized individually. • Adjust the annealing temperature in 2–3 °C increments or use a gradient PCR to find the optimal annealing temperature. • Increase the number of amplification cycles. If currently using 25–30 cycles, increase the cycle number to 35–40. • For complex templates like human genomic DNA, increase the initial denaturation time by 1–2 minutes and/or increase the denaturation temperature to 95 °C to overcome denaturation difficulties. • Check concentration of input template. For complex templates like intact eukaryotic genomic DNA, 1000 genome copies may be required for amplification of difficult targets. For highly concentrated templates, such as purified plasmid, consider diluting 1:1000 to improve amplification. • Assess DNA quality to ensure absence of PCR inhibitors in sample. If presence of inhibitors is suspected, DNA can be diluted 1:10–1:100. Alternatively, lysis and DNA purification can be performed using the GenElute™ genomic DNA miniprep kits. • Refer to "PCR-Enhancing Additives" section of the Technical Guide to improve amplification. • If yield is too low for downstream applications, increase the reaction volume to 50–75 µL.
Amplification of nonspecific product(s)	• Raise the annealing temperature in 2–3 °C increments or use a gradient PCR to find the optimal annealing temperature. Raising the temperature improves the specificity of binding by the primers; however, it may also result in reduced binding and extension of the primers.[6,7] If raising the annealing temperature causes reduced yield of the specific product without eliminating side reaction products, it may be necessary to redesign the primers to improve specificity. • Take precautions to avoid crossover contamination of PCR with both specific and nonspecific PCR products, including primer-dimer artifacts.[17] • The use of more than 5% v/v DMSO with JumpStart™ *Taq* is not recommended as it may interfere with the enzyme-antibody complex. Other co-solvents, salts, and extremes in pH can also reduce the affinity of the JumpStart™ *Taq* antibody for the *Taq* DNA Polymerase and compromise its effectiveness for hot start PCR.

References

1. Dieffenbach, C., and Dveksler, G., (eds) PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1995.
2. Oksana Shynlova et al. *Journal of cellular and molecular medicine, 17(2), 311–324 (2013-02-06)*
3. Oksana Shynlova et al. *Journal of cellular and molecular medicine, 17(1), 90–102 (2012-12-05)*
4. Khrystyna Levytska et al. *PPAR research,* 2014, 637251–637251 (2014-04-09)
5. Xiang Zhao et al*. The Journal of biological chemistry*, 286(26), 23200–23213 (2011-04-30)
6. Devika Sharanya et al. *Evolution & development*, 17(1), 34–48 (2015-01-30)
7. Jan Gravemeyer et al. *Oncogene*, 41(1), 37–45 (2021-10-21)
8. Dieffenbach, CW, and Dveksler, GS. PCR Primer: A Laboratory Manual. Cold Spring Harbor Laboratory Press, (2003).
9. Green MR, Sambrook J, Cold Spring Harb Protoc. 6, 436–456(2019).
10. Markoulatos, P., et al., J. Clin. Lab. Anal. 16, 47–51 (2002).
11. Paabo, S., et al., Nucleic Acids Res., 16, 9775–9787 (1988).
12. Sarkar, G., et al., Nucleic Acids Res., 18, 7465 (1990).
13. Cheng, S., et al., Proc. Natl. Acad. Sci. USA, 91, 5695–5699 (1994).
14. Grunenwald H. PCR Protocols. Methods in Molecular Biology™. 226, 89–99 (2003).
15. Chou, Q., Nucleic Acids Res., 20, 4371 (1992).
16. Rees, W. A., et al., Biochemistry, 32, 137–144 (1993).
17. Kwok S., and Higuchi R., Nature 339, 237–238 (1989).

Product Ordering

Description	Catalogue Number
JumpStart™ *Taq* DNA Polymerase	D4184
Deoxynucleotide Mix, 10 mM	D7295
Deoxynucleotide Mix, 25 mM	D7297
Betaine solution	B0300
Mineral Oil	M5904 M8662
1 kb DNA Ladder	D0428
Custom ordered primers specific to gene target	OLIGO
GenElute™-E Single Spin DNA Cleanup Kit	EC600
GenElute™ PCR Clean-Up Kit	NA1020
GenElute™ Gel Extraction Kit	NA1111
Precast Agarose Gels	P6222 P5472 P6097 P5972 P5722
1 kb DNA Ladder	D0428
Water, Microbial DNA-free	MBD0025
Nuclease-Free Water, for Molecular Biology	W4502
RED*Taq*® Ready Mix	P0982
Glycerol-free JumpStart™ *Taq* DNA Polymerase	D9310
DMSO	D8418
Single strand binding protein	S3917

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/office.

23346362 Rev 02/26