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Product Information

LuminoCt® SYBR® Green qPCR ReadyMix™

Catalog Number L6544
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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 that 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.

- Designed specifically for rapid, two-step qPCR protocols that deliver results in as little as 25 minutes (see procedure).
- LuminoCt ReadyMixes require the use of small amplicons (<200 bp) for optimal results. The ReadyMixes are compatible with commercial primer sets, including TaqMan® Assays.
- The hot start mechanism using the JumpStart Taq antibody, which prevents non-specific product formation, allows assembled PCR reactions to be placed at room temperature for up to 4 hours without compromising the performance.
- When performing large numbers of PCR reactions, LuminoCt SYBR Green qPCR 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

- LuminoCt SYBR Green qPCR ReadyMix, Catalog Number 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, Catalog Number R4526. Optional, for use with instruments compatible with an internal reference dye (e.g. ABI and Stratagene).

Reagents and Equipment Required But Not Provided

- Water, PCR Reagent, Catalog Number W1754
- Primers
- DNA template
- Dedicated pipettes
- PCR pipette tips
- Plates and optical caps for specific thermal cycler
- Quantitative (or real time) thermal cycler, with standard or Peltier block

Precautions and Disclaimer

LuminoCt SYBR Green qPCR ReadyMix is for R&D use only, not for drug, household, or other uses. Please consult the Material 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. It can also be stored at 2–8 °C for up to 6 months, so there is no waiting for the reaction components to thaw.

Procedure
LuminoCt SYBR Green qPCR ReadyMix has been formulated to give robust amplification under a variety of conditions. For reactions that will be run many times it is worthwhile to optimize primer concentrations to increase target specificity and sensitivity. In addition, on rare occasions, we have found that the optimal concentrations of template DNA, $MgCl_2$, KCl, and PCR adjuncts can be target specific. Additional components ($MgCl_2$, dNTP, or betaine) may be added to the template/primer mixture, although this is not required for the vast majority of applications. The following procedure serves as a reference.

Note: DMSO (up to 5% v/v) is compatible with this system. However, other co-solvents, 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.

The use of primer design software is highly recommended as well-constructed, high-specificity primers are necessary to obtain good qPCR data. In addition, it is essential that the primers used for qPCR define an amplicon of 90-200 bp in length. Commercially available primer and probe sets, including TaqMan Assays, are compatible with LuminoCt ReadyMix.

1. Add the following reagents to a 0.2 ml or 0.5 ml thin-walled microcentrifuge tube or plate well.

Volume (µL)			Reagent	Final Concentration
25	12.5	10	2X LuminoCt SYBR Green qPCR ReadyMix	1X LuminoCt SYBR Green qPCR ReadyMix
0.4	0.2	0.16	25 µM Forward primer	0.1-0.8 µM *
0.4	0.2	0.16	25 µM Reverse primer	0.1-0.8 µM *
x	x	z	Template DNA	varied, ~pg-ng
x′	y′	z′	100X ROX internal reference dye	0.1-6.0X. As required by instrument §
q.s.	q.s.	q.s.	Water	
50	25	20	Total Volume	

* These are acceptable ranges for a reaction, with the proposed additions representing 0.2 µM for each primer.

§ Reference dye is unnecessary when using many qPCR instruments, and can be excluded. Quantitative PCR instruments requiring reference dye are listed below, including recommended default concentrations. It may be necessary to increase the concentration of reference dye to obtain optimal results. **Review appropriate troubleshooting guidelines prior to first experiments.**

qPCR instrument	Reference Dye
ABI 7500	0.1X
Stratagene MX4000, MX3005, MX3000	0.1X
ABI 7900HT, 7700, 7300, 7000 and StepOne	1.0X

Note: A template-primer master mix for each dilution of template is recommended when performing multiple PCR reactions.

2. Mix gently by vortexing and briefly centrifuge to collect all components at the bottom of the tube

Note: Optimal cycling parameters vary with PCR composition and thermal cycler. It may be necessary to optimize the cycling parameters to achieve maximal PCR efficiency and sensitivity.

Recommended cycling parameters for <200 bp amplicons §

Initial denaturation ¤	95 °C	20 sec
40 cycles:		
Denaturation	95 °C	3 sec
Annealing/ Extension*	60 °C	15-30 sec
Hold	4 °C	

§ The ReadyMix is also compatible with the fast cycling protocols of the ABI 7900HT Fast, ABI 7500 Fast, ABI StepOne, Bio-Rad CFX96 and CFX384, Roche Lightcycler® 480 and Eppendorf Mastercycler ep realplex.

¤ Amplification of difficult templates, such as human genomic DNA, may benefit from longer (up to two minute) initial denaturation times

* Optimal extension times vary with amplicon length. In general, an extension step of 20 sec is sufficient for all amplicons under 200 bp in length. It may be necessary to increase the length of this step to conform to the minimum extension time of certain instruments or to ensure complete replication of certain amplicons.

References

1. Dieffenbach, C., and Dveksler, G., (eds) PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1995.
2. Rees, W.A., *et al.*, Betaine can eliminate the base pair composition dependence of DNA melting. *Biochemistry*, **32,** 137-144 (1993).

Troubleshooting Guide

Problem	Possible Cause	Solution
No PCR product is observed.	A PCR component is missing or degraded.	A positive control should always be run to insure components are functioning. A checklist is also recommended when assembling reactions.
	SYBR is degraded.	Run an agarose gel to analyze the reaction product. If an appropriately sized single band is evident, then detection is faulty. SBYR Green I is light sensitive and must be protected.
	The annealing temperature is too high.	Decrease the annealing temperature in 2-4 °C increments.
	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. There may be no template due to extraction or purification failure.
	Primers are not designed optimally.	Check primer set by running a dilution series on a known template. Reorder or redesign as needed.
	The initial denaturation temperature is too long.	Remove the activation step. JumpStart Taq may be degraded with long (>3 min) initial denaturation times.
	Target template is complex.	In most cases, inherently complex 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.[2]
	Reference dye is mismatched	For Rn (normalized fluorescence) plots turn off reference dye. Alternatively, view the raw fluorescence of qPCR amplification plot. Removing normalization often restores plots to the expected shape, allowing the calculation of more reasonable Ct values. Alternatively, one may wish to titrate the reference dye in the reaction. See suggestions in final troubleshooting section.
PCR efficiency is too low (<80%)	The annealing temperature is too low.	Increase the annealing temperature in increments of 2-3 °C.
	Template contains inhibitors	Run a standard curve (log [DNA] vs Ct). If the curve is non-linear at high DNA/cDNA concentrations either revise the DNA/cDNA purification or limit template concentrations to linear range.
	The primers are not designed optimally.	Run a melt curve or agarose gel to check for the presence of multiple amplicons.
	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.
	The initial denaturation temperature is too long.	Remove the activation step. JumpStart Taq may be degraded with long (>3 min) initial denaturation times.

Troubleshooting Guide (continued)

Problem	Possible Cause	Solution
PCR efficiency is too high.	Multiple loci hybridize to the primer set.	Run a melt curve or agarose gel to check for the presence of multiple amplicons. Alternatively, for a sequenced target genome use the NCBI program e-PCR looking for multiple amplicons.
Technical replicates return widely varied Ct values or data gives uninter-pretable amplifi-cation curves	Pipetting errors cause the fluctuation	Prepare large volume of complete mix and aliquot this into separate reactions. If the variance persists, see below.
	Instrument requires an internal reference dye	Add reference dye such as R4526 (100X Reference Dye) as required for the specific quantitative PCR instrument (below).
	Reference dye is mismatched	For Rn (normalized fluorescence) plots turn off reference dye. Alternatively, view the raw fluorescence of qPCR amplification plot. Removing normalization often restores plots to the expected shape, allowing the calculation of more reasonable Ct values. If normalization is desired, the optimal amount of reference dye must be determined by titration. As a guide, for protocols run on an ABI7500 or Stratagene instrument, it was found that internal reference dye is only needed at a concentration of 0.1X, while on other ABI instruments, internal reference dye is needed at a concentration of 1.0X. In certain instances, it was found to be necessary to increase the concentration of reference dye for optimal results. As a rule, users should not exceed a reference dye concentration of 1.0X when using an ABI 7500 or Stratagene instrument and 6.0X when using other ABI instruments.

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