

Clyde R. Brown, Erinn von Rein and Erik R. Eastlund

Sigma-Aldrich Biotechnology, 2909 Laclede Ave., St. Louis, MO 63103 USA

Abstract

Sigma-Aldrich has developed quantitative PCR and RT-PCR products to meet a wide range of researcher needs. These products are designed to work with different detection chemistries and instruments for quantitative PCR and RT-PCR, as each requires different product formulations. JumpStart™ Taq ReadyMix™ for quantitative PCR is ideal for high throughput and offers maximum flexibility in detection method since no detection chemistry has been incorporated into the formulation. For contamination control concerns, JumpStart™ Taq ReadyMix™ with dUTP incorporates dUTP in the place of TTP and may be used on both capillary and noncapillary real-time thermal cyclers. Universal detection chemistry is incorporated into several SYBR® Green I dye-containing products which have recently become available. Sigma-Aldrich offers a specially formulated SYBR Green quantitative PCR ReadyMix for use on capillary-based platforms, such as the Roche LightCycler™, while a second SYBR Green quantitative PCR ReadyMix is formulated for plate/tube real-time thermal cyclers. Finally, Sigma-Aldrich's SYBR Green RT-PCR kit is suitable for gene expression studies using either real-time instrument platform. Data is presented showing the sensitivity, application and capabilities of this new series of Sigma-Aldrich products.

Sigma-Aldrich Quantitative PCR ReadyMixes for Sequence-Specific Fluorescent Detection Chemistries

Sigma-Aldrich offers two quantitative PCR ReadyMixes that do not contain a fluorescent detection method. These two mixes are compatible for use with many sequence-specific formats as well as non-specific intercalating dyes. Dual-labeled probes, Molecular Beacons, or double stranded binding dyes such as SYBR Green I can all be individually optimized for use with these two ReadyMixes.

JumpStart Taq ReadyMix for quantitative PCR, Product Code D 7440, is formulated for use on plate/tube real-time instruments. Sigma's ReadyMix formulations contain JumpStart Taq DNA polymerase, all four nucleotides (dATP, dCTP, dGTP and TTP), buffers, magnesium, and stabilizers in a convenient 2X concentrate.

All of Sigma's ReadyMixes for real-time PCR and RT-PCR utilize JumpStart Taq DNA polymerase, an antibody-inactivated enzyme. This reduces nonspecific priming and amplification since the JumpStart Taq antibody renders the Taq DNA polymerase inactive at room temperature. During the first denaturation cycle of PCR, the antibody dissociates from the enzyme and full activity is restored. No special preparations or extensive reactivation steps are required with JumpStart Taq DNA polymerase.

Quantitative PCR was performed using human genomic DNA. The template was diluted 10-fold in subsequent wells; concentrations were 10 ng, 1 ng, 0.1 ng, and 0.01 ng. A TaqMan® probe and primers specific for a 250 bp PCR product of the β-actin gene were used with Sigma's JumpStart Taq ReadyMix for Quantitative PCR or a master mix from Supplier A. Final magnesium concentration was adjusted to 3.5 mM. Thermal cycling conditions were those recommended by Supplier A. The JumpStart Taq ReadyMix (in red) has better amplification efficiency (Figure 1), resulting in lower CT values than Supplier A (in blue) (Figure 2).

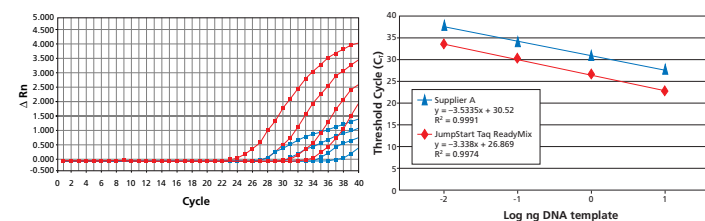


Figure 1

Figure 2

JumpStart Taq ReadyMix with dUTP, Product Code D 9191, incorporates dUTP in place of TTP to facilitate carryover prevention. Contamination is the single most important obstacle to using PCR reliably for diagnostic purposes. This nucleotide mixture provides the ability to eliminate contaminating PCR products by use of a Uracil-DNA glycosylase (UNG). This dUTP containing PCR ReadyMix is formulated for use on both plate/tube and capillary-based real-time instruments.

Three experiments were performed to compare the efficiency of JumpStart Taq ReadyMix with dUTP to that of competitor master mixes. The first comparison was using a master mix from Supplier A. Human genomic DNA was used as the template and diluted 10-fold in subsequent wells; concentrations were 30 ng, 3 ng, 0.3 ng, 0.03 ng and 0.003 ng. A β-actin TaqMan® probe and primers provided by supplier A were used. Thermal cycling conditions were those recommended by Supplier A. The JumpStart Taq ReadyMix with dUTP gave a higher response with better PCR efficiency than Supplier A's master mix (Figure 3).

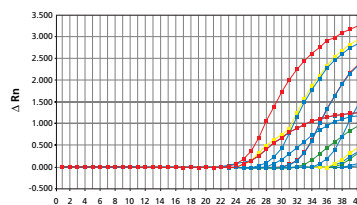


Figure 3

Two additional comparisons were performed using master mixes from competitors R and Q and were run on a Roche LightCycler real-time thermal cycler. The detection method for these two additional quantitative PCR comparisons used the non-specific SYBR Green I intercalating dye. Human genomic DNA was used as the template and diluted 10-fold in subsequent wells; concentrations were 30 ng, 3 ng, 0.3 ng, 0.03 ng and 0.003 ng. Specific primers generating a 250 bp PCR product of the β-actin gene were used. An additional 15 minute incubation was added to the cycling program for the master mix from Supplier Q and an additional 10 minute incubation was added to the cycling program for the master mix from Supplier R to activate the Taq DNA polymerase (data not shown). Sigma's JumpStart Taq ReadyMix with dUTP has a PCR efficiency higher than the master mix from Supplier R and an efficiency comparable to the master mix from Supplier Q (Table 1).

Table 1. Comparison of JumpStart Taq ReadyMix with dUTP with master mixes from three competitors.

	Standard Curve Slope	R ² value	PCR Efficiency $E = (10^{-1/\text{slope}}) - 1$ 100% efficiency will generate a slope of -3.32.	Instrument Platform
JumpStart Taq ReadyMix with dUTP	-3.496	0.978	0.94	ABI 7700
Supplier A master mix	-3.912	0.998	0.80	
JumpStart Taq ReadyMix with dUTP	-3.422	1	0.96	Roche LightCycler
Supplier R master mix	-3.589	1	0.90	
JumpStart Taq ReadyMix with dUTP	-3.583	1	0.92	Roche LightCycler
Supplier Q master mix	-3.579	1	0.92	

An experiment was performed to confirm suitability for use in carryover prevention by degrading JumpStart Taq ReadyMix with dUTP amplicon with uracil DNA glycosylase. Uracil-containing DNA from a prior amplification was spiked into PCR reactions prepared with JumpStart Taq ReadyMix with dUTP. Prior to amplification this uracil-containing DNA contamination was degraded by uracil DNA glycosylase. Up to 28 pg of uracil-containing DNA was cleared in this experiment (data not shown).

A vial of reference dye for use in ABI Detection Systems and a vial of 25 mM MgCl₂ for further optimization are provided with both JumpStart Taq ReadyMix and JumpStart Taq ReadyMix with dUTP.

Sigma-Aldrich

Quantitative PCR ReadyMixes with SYBR Green

Sigma-Aldrich offers two quantitative PCR ReadyMixes that contain SYBR Green I dye as the fluorescent detection method. SYBR Green I fluorescent dye binds selectively to double-stranded DNA, with no significant fluorescence in the presence of single-stranded DNA. Detection of the DNA is monitored by measuring the increased fluorescence throughout the PCR cycles. SYBR Green I reporter has high sensitivity, is easy to use and is less expensive than sequence-specific fluorescent probes. It is the most common nonspecific detection method used for quantitative PCR/RT-PCR.

These two SYBR Green PCR ReadyMixes are recommended for single target real-time amplification experiments. They can also be used for optimization of primer design using unlabeled primers prior to manufacture of fluorescent-labeled probes. Both of these mixes employ the performance enhancements of JumpStart Taq antibody for hot start PCR in a convenient, easy-to-use 2X concentrate.

SYBR Green JumpStart Taq ReadyMix, Product Code S 4438, is recommended for use on plate/tube real-time instruments. A vial of reference dye is supplied for use in ABI Detection Systems along with a vial of 25 mM MgCl₂ for further optimization.

Quantitative PCR was performed on pBac-2cp. Initial template copy number was 10⁶ and was diluted 10-fold in subsequent wells. Threshold cycles (C_T) were determined using the ABI Prism 7700 Sequence Detection Software. SYBR Green JumpStart Taq ReadyMix has a linear response over seven orders of magnitude (Figure 5).

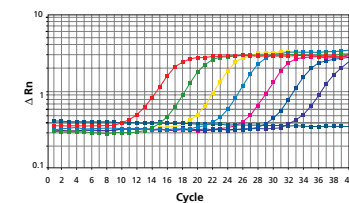


Figure 4

SYBR Green JumpStart Taq ReadyMix, Capillary Formulation, Product Code S 1816, is specially designed for use in capillary based instruments, such as the Roche LightCycler real-time thermal cycler. A vial of 25 mM MgCl₂ is provided for further optimization.

Quantitative PCR was performed using human genomic DNA. Human genomic DNA template was diluted 10-fold in subsequent wells; concentrations were 30 ng, 3 ng, 300 pg, 30 pg, 3 pg. Sigma's SYBR Green Taq ReadyMix, Capillary Formulation was used with specific primers to amplify a 250 bp PCR product of the β-actin gene.

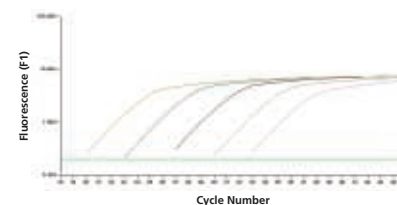


Figure 5

SYBR Green Quantitative RT-PCR Kit

The SYBR Green quantitative RT-PCR kit combines eAMV™ RT, an enhanced avian reverse transcriptase, JumpStart Taq DNA polymerase and SYBR Green I fluorescent dye in a one step RT-PCR kit designed for measurement of gene expression. eAMV-RT has the ability to transcribe through difficult secondary structure at elevated temperature (up to 65 °C). JumpStart Taq DNA polymerase uses Jumpstart Taq antibody to inactivate the enzyme below 70 °C, preventing primer-dimer and non-specific product formation. Since SYBR Green I dye will detect all nonspecific quantitative RT-PCR product formation, well-designed primers are recommended for this system to ensure the highest possible specificity.

The kit has been optimized for use with both plate/tube real time instruments and with the Roche LightCycler capillary instrument. A reference dye is provided in a separate vial to be used with ABI Detection Systems. A vial of 25 mM MgCl₂ is also provided for further optimization.

Quantitative SYBR Green one-step RT-PCR was performed on human total RNA from cell line HeLa-S3. The total RNA was DNase treated using Sigma's DNase kit (Product Code AMP-D1) and diluted 10-fold in subsequent capillaries; concentrations were 500 ng, 50 ng, 5 ng, 500 pg, 50 pg, 5 pg. Primers specific for a β-actin 187 bp RT-PCR product were used along with SYBR Green Taq ReadyMix for quantitative RT-PCR and eAMV reverse transcriptase. Sigma's SYBR Green RT-PCR kit shows a linear response over six orders of magnitude with sensitivity down to 5 pg of total RNA template (Figure 6).

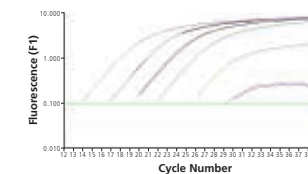


Figure 6

Conclusions

1. JumpStartTaq ReadyMix for quantitative PCR has better amplification efficiency resulting in lower C_T values than master mixes from Supplier A.
2. JumpStart Taq ReadyMix with dUTP gives comparable performance results when compared with three leading competitors on both capillary and noncapillary formats.
3. SYBR Green JumpStart Taq ReadyMix has a linear response over 7 orders of magnitude.
4. The SYBR Green quantitative RT-PCR kit demonstrates a linear response over 6 orders of magnitude with high sensitivity.

The Sigma-Aldrich quantitative PCR/RT-PCR product line offers competitive performance with fluorescent detection method flexibility, the option of carryover contamination prevention and instrument flexibility.

Purchase of these products is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process for research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e., and authorized thermal cycler.

SYBR is a registered trademark of Molecular Probes, Inc. Licensed from Molecular Probes, Inc. TaqMan is a registered trademark of and LightCycler is a trademark of Roche Molecular Systems, Inc. JumpStart Taq antibody is licensed under U.S. Patent No. 5,338,671 and 5,587,287, and corresponding patents in other countries.