

Quantitative PCR

Quantitative PCR Product Listing

Catalog Number	Product Description	Page
SYBR-based		
S4438	SYBR Green JumpStart Taq ReadyMix	20
S1816	SYBR Green JumpStart Taq ReadyMix, Capillary Formulation	22
S5193	SYBR Green Jumpstart Taq ReadyMix without MgCl ₂	20
S9194	SYBR Green ReadyMix for High Throughput Quantitative PCR	24
QR0100	SYBR Green Quantitative RT-PCR Kit	25
Probe-based		
D7440	JumpStart Taq ReadyMix for Probe-based qPCR applications	23
D6442	JumpStart Taq ReadyMix for High Throughput Quantitative PCR	24
QR0200	Quantitative RT-PCR ReadyMix for Probe-based applications	26
PPD1	PCR Plate Detection Kit (sufficient for 480 detection reactions)	27
R4526	Reference Dye for Quantitative PCR	27

SYBR® Green JumpStart Taq ReadyMix

Real time detection in a complete reagent

SYBR Green JumpStart Taq ReadyMix for Quantitative PCR combines the advantages of a hot start enzyme with a ready-to-use mix, making it the ideal choice for high throughput, quantitative PCR. The ReadyMix includes the fluorescent dye SYBR Green I, JumpStart Taq DNA Polymerase, 99% pure deoxynucleotides and buffer in an optimized 2× concentrate.

SYBR Green JumpStart Taq ReadyMix is recommended for single product real time amplification experiments. It may also be used for evaluation of primer sequences prior to manufacture of fluorescent-labeled primers. Fluorescent-labeled primers are not recommended for use with SYBR Green I dye.

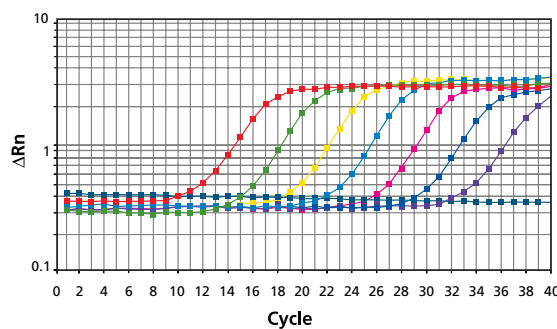
SYBR Green I, a commonly used fluorescent DNA binding dye, binds all double-stranded DNA and thus product accumulation is detected by measuring the increase in fluorescence throughout the cycle. SYBR Green I has an excitation and emission maxima of 494 nm and 521 nm, respectively.

JumpStart Taq Polymerase is an antibody inactivated hot start enzyme designed to minimize non-specific amplification while increasing target yield resulting in more accurate Ct values and improved standard curve for absolute sample quantitation.

To prepare a reaction, add 25 µl of ReadyMix to primers, template and water for a final reaction volume of 50 µl.

Sigma's Reference Dye for Quantitative PCR is included with this ReadyMix for normalization of the reaction data. The dye has a maximum excitation of 586 nm, and a maximum emission of 605 nm. The instrument settings for ROX Reference Dye are suitable for the measurement of the Reference Dye for Quantitative PCR.

Ct Values for the Lambda Amplicon Using SYBR Green JumpStart Taq ReadyMix



Ct values for the lambda amplicon using SYBR Green JumpStart Taq ReadyMix. Quantitative PCR (qPCR) was performed on pBac-2cp. Initial template copy number was 10⁶ and was diluted 10-fold in subsequent wells. Threshold cycles (Ct) were determined using the ABI PRISM 7700 Sequence Detection software, and were found to be 15.304 (10⁶), 18.848 (10⁵), 22.883 (10⁴), 26.208 (10³), 29.821 (10²), 33.398 (10¹), 37.038 (10⁰), and 40 (0).

Components: SYBR Green JumpStart Taq ReadyMix
Reference Dye for Quantitative PCR
Separate Vial of 25 mM MgCl₂ Included in S5193

Unit definition: One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C

Concentration: 1.5 units per reaction (50 µl reaction volume)

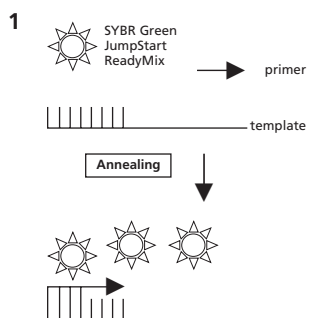
Storage: -20 °C
Shipped in wet ice

Ordering Information

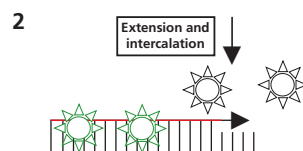
Cat. No.	Product Description	Quantity
S4438	SYBR Green JumpStart Taq ReadyMix	100 reactions 500 reactions
S5193	SYBR Green JumpStart Taq ReadyMix without MgCl ₂	100 reactions 400 reactions

Mechanism of SYBR Green I in qPCR

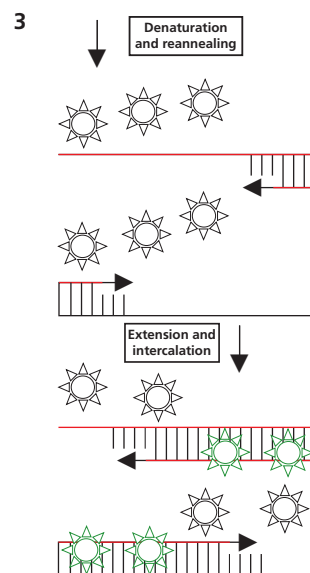
1) Set up your PCR reaction with the SYBR Green JumpStart Taq ReadyMix. The mix contains enzyme, dNTPs, buffer and SYBR Green I dye. Just add your primers and template.



2) As the reaction progresses, double-stranded products are generated. The SYBR Green I dye intercalates into these products and begins to fluoresce.



3) When enough products have accumulated the fluorescence rises above background. This is called the threshold cycle or Ct. The Ct value is used to quantify the starting amount of template.



Quantitative PCR

SYBR® Green JumpStart™ Taq ReadyMix™

Capillary Formulation

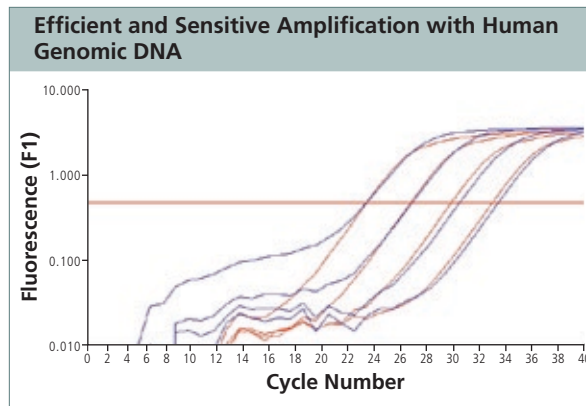
SYBR Green JumpStart Taq ReadyMix, Capillary formulation combines the advantages of a hot start enzyme, JumpStart Taq, in a 2× concentrate ReadyMix specifically designed for use with capillary instruments, such as the Roche LightCycler® real-time thermal cycler. SYBR Green JumpStart Taq ReadyMix is an optimized formulation containing SYBR Green I dye, JumpStart Taq DNA Polymerase, 99% pure deoxynucleotides, buffer and stabilizers.

SYBR Green JumpStart Taq ReadyMix is recommended for single product real time amplification experiments. Fluorescent-labeled probes are not recommended for use with SYBR Green I dye.

SYBR Green I binds all double-stranded DNA and detection is monitored by measuring the increase in fluorescence throughout the cycle.

JumpStart Taq Polymerase, an antibody inactivated hot start enzyme, is designed to minimize non-specific amplification while increasing target yield resulting in more accurate Ct values and an improved standard curve for absolute sample quantitation.

To prepare a reaction, 10 µl of ReadyMix is added to primers, template and water for a final reaction volume of 20 µl. Once the reaction has reached 70 °C, the complex dissociates and the polymerase becomes fully active. No special preparations or protocol changes are required.



Efficient and sensitive amplification with human genomic DNA.

Quantitative PCR was performed on human genomic DNA using the Roche LightCycler. The template was diluted 10-fold in subsequent wells with concentrations of 30 ng to 30 µg. Sigma's SYBR Green Taq ReadyMix, capillary formulation (in red) has comparable sensitivity and efficiency to supplier R's SYBR Green master mix (in blue).

Components: SYBR Green JumpStart Taq ReadyMix Reference Dye for Quantitative PCR
Separate Vial of 25 mM MgCl₂ Included in [S5193](#)

Unit definition: One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C

Storage: -20 °C
Shipped in wet ice

Ordering Information

Cat. No.	Product Description	Quantity
S1816	SYBR Green Taq ReadyMix, Capillary Formulation	100 reactions 200 reactions
S5193	SYBR Green JumpStart Taq ReadyMix without MgCl ₂	100 reactions 400 reactions

JumpStart™ Taq ReadyMix™ for Quantitative PCR

For probe-specific real time PCR applications

JumpStart Taq ReadyMix for Quantitative PCR combines the advantages of a hot start enzyme with a ready-to-use mix for high throughput, quantitative PCR (qPCR). It does not contain a fluorescent detection method, which makes it compatible for use with many formats. Dual-labeled probes, Molecular Beacons, or double-stranded binding dyes such as SYBR Green I dye can all be individually optimized for use with this ReadyMix.

The ReadyMix contains JumpStart Taq DNA Polymerase, 99% pure deoxynucleotides and buffer in an optimized 2× concentrate. JumpStart Taq Polymerase, an antibody inactivated hot start enzyme, is designed to minimize non-specific amplification while increasing target yield. Unlike other hot start methods (i.e., chemical inactivation), JumpStart Taq Polymerase does not require a pre-incubation step prior to cycling because polymerase activity is fully restored during the first denaturation cycle of the PCR reaction. To prepare a reaction, add 25 µl of the ReadyMix to primers, template, detection component and water for a total reaction volume of 50 µl.

Sigma's Reference Dye for Quantitative PCR is included with this ReadyMix for normalization of the reaction data. The dye has a maximum excitation of 586 nm, and a maximum emission of 605 nm. The instrument settings for ROX reference dye are suitable for the measurement of the Reference Dye for Quantitative PCR. A tube of 25 mM MgCl₂ is provided for easy optimization of the qPCR reaction.

Features and Benefits

- Minimize non-specific amplification while increasing target yield and specificity, both of which result in lower, more accurate Ct values
- Compatible with a variety of fluorescent detection methods including dual-labeled probes and Molecular Beacons, recommended for multiplex comparative studies
- Designed for use with either plate/tube real time thermal cyclers or capillary instruments

Superior Amplification Efficiency with Lower Ct

Figure 1.

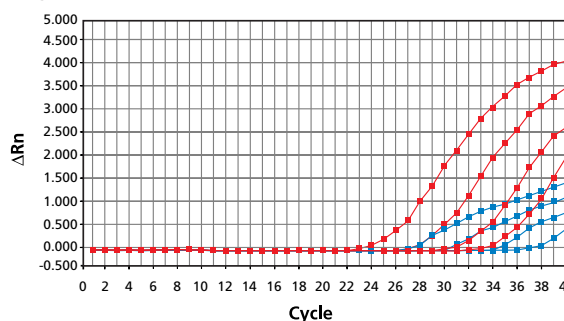
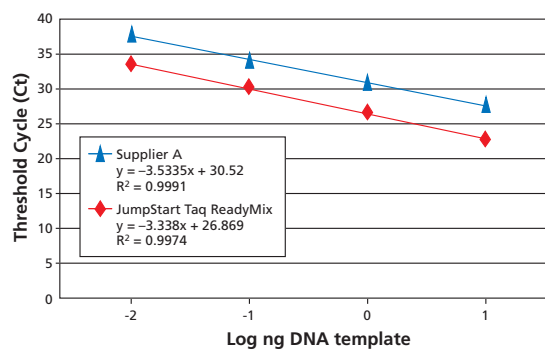


Figure 2.



Superior amplification efficiency with lower Ct. Quantitative PCR was performed on 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).

Components: JumpStart Taq ReadyMix
25 mM MgCl₂
Reference Dye for Quantitative PCR

Unit definition: One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C

Concentration: 1.5 units per reaction (50 µl reaction volume)

Storage: -20 °C
Shipped in wet ice

Ordering Information

Cat. No.	Product Description	Quantity
D7440	JumpStart Taq ReadyMix for Quantitative PCR	100 reactions 400 reactions

Quantitative PCR

JumpStart™ Taq ReadyMix™ for High Throughput Quantitative PCR

JumpStart Taq ReadyMix for High Throughput Quantitative PCR combines the advantages of a hot start enzyme with the convenience of an easy-to-use reaction mixture. The 2× concentrated ReadyMix contains JumpStart Taq DNA polymerase, 99% pure deoxynucleotides, reaction buffer, and an internal reference dye for ABI and other real time instrument applications. To set up the reaction simply add an equal volume of the ReadyMix to a 2× mixture of DNA template, primers and fluorescent probe.

- Internal reference dye incorporated into ReadyMix for quick set-up
- High throughput ReadyMix contains all necessary reagents except probe, primers and template DNA
- Hot Start PCR for more accurate Ct values and improved quantitation

Ordering Information

Cat. No.	Product Description	Quantity
D6442	JumpStart Taq ReadyMix for High Throughput Quantitative PCR	400 reactions 2,000 reactions

SYBR® Green ReadyMix for High Throughput Quantitative PCR

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. The 2× concentrated ReadyMix contains the SYBR detection fluor, JumpStart Taq DNA polymerase, 99% pure deoxynucleotides, reaction buffer, and an internal reference dye for ABI and other real time instrument applications. To set up the reaction simply add an equal volume of the ReadyMix to a 2× mixture of DNA template and primers.

- SYBR Green I dye for nonspecific sequence detection in quantitative PCR
- High throughput ReadyMix contains all necessary reagents, except primers, and template DNA
- Hot Start PCR for more accurate Ct values and improved quantitation

Ordering Information

Cat. No.	Product Description	Quantity
S9194	SYBR Green ReadyMix for High Throughput Quantitative PCR	400 reactions 2,000 reactions

SYBR® Green Quantitative RT-PCR Kit

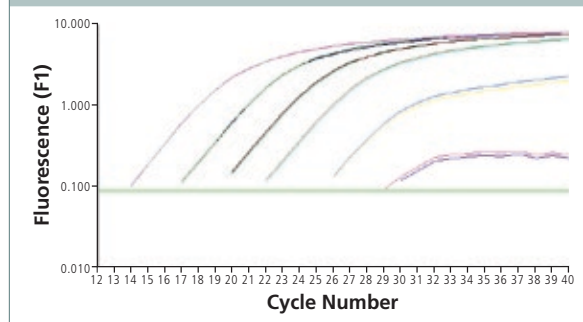
The SYBR Green Quantitative RT-PCR Kit combines M-MLV Reverse Transcriptase, JumpStart Taq DNA Polymerase and SYBR Green I fluorescent dye in a one-step RT-PCR kit designed for the quantitative analysis of gene expression. The ReadyMix includes SYBR Green I dye, JumpStart Taq DNA Polymerase, 99% pure deoxynucleotides, buffer and stabilizers, and is provided as a 2× concentrate for convenience. JumpStart Taq DNA Polymerase uses JumpStart Taq antibody to inactivate the enzyme below 70 °C, preventing primer dimers and non-specific product formation. The double-stranded DNA-specific fluorescent SYBR Green I reporter has high sensitivity, is easy to use and is less expensive than sequence-specific fluorescent probes.

The SYBR Green Quantitative RT-PCR Kit delivers high specificity and reproducibility. Since SYBR Green I dye will detect all non-specific 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 in ABI Detection Systems.

Features and Benefits

- One-tube reaction setup minimizes hands-on time and contamination risk
- Minimize non-specific amplification while increasing target yield and specificity, both of which result in lower, more accurate Ct values
- SYBR based detection allows quantitation of all double-stranded DNA. Specificity is greatly enhanced by the incorporation of JumpStart Taq. Optimized to help you achieve superior results, our RT-PCR Kit is compatible with tube, plate, and capillary-based instruments

Sensitive Quantitative RT-PCR Using Sigma's SYBR Green Quantitative RT-PCR Kit



Sensitive quantitative RT-PCR using Sigma's SYBR Green Quantitative RT-PCR Kit. Quantitative SYBR Green RT-PCR was performed in duplicate on human total RNA from cell line HeLa-S3. The total RNA was diluted 10-fold in subsequent capillaries with concentrations of 500 ng to 5 pg. Primers specific for a β -actin 187 bp RT-PCR product were used for amplification.

Components: SYBR Green Taq ReadyMix for Quantitative RT-PCR
M-MLV Reverse Transcriptase
10× PCR Buffer
25 mM MgCl₂
Reference Dye for Quantitative PCR

Storage: -20 °C
Shipped in wet ice

Ordering Information

Cat. No.	Product Description	Quantity
QR0100	SYBR Green Quantitative RT-PCR Kit	1 kit

Quantitative PCR

Quantitative Reverse Transcriptase PCR ReadyMix for Probe-Based Applications

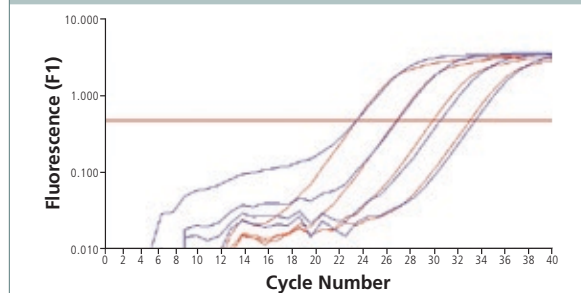
Quantitative Reverse Transcriptase PCR (qRT-PCR) provides a highly sensitive method for the quantitative analysis of gene expression. Sigma's qRT-PCR ReadyMix combines M-MLV reverse transcriptase and JumpStart Taq with a ready-to-use mix specifically designed for probe-based qRT-PCR. Sigma's qRT-PCR ReadyMix is specially formulated to help you achieve superior results regardless of difficult secondary structure or chosen fluorescent detection chemistry.

qRT-PCR ReadyMix for probe-based applications is a 2× concentrate blend of JumpStart Taq, 99% pure dNTPs, buffer, glass passivator and stabilizers. The ReadyMix is also packaged with a separate vial of M-MLV RT, 25 mM MgCl₂, 10× PCR Buffer, and Reference Dye for normalization of the reaction data.

Features and Benefits

- One-tube reaction setup minimizes hands-on time and contamination risk
- Minimize non-specific amplification while increasing target yield and specificity, both of which result in lower, more accurate Ct values
- Compatible with a variety of fluorescent detection methods including dual-labeled probes and Molecular Beacons, Sigma's ReadyMix is also formulated for use on tube, plate, and capillary-based instruments

Superior Sensitivity and Specificity with Sigma's qRT-PCR ReadyMix



Superior sensitivity and specificity with Sigma's qRT-PCR ReadyMix.

Quantitative RT-PCR was performed in duplicate on total RNA from the HeLa S3 cell line. Total RNA was DNase treated and diluted 10-fold in subsequent capillaries. Forward and reverse primers specific for c-Myc were used for amplification. Measurements were made using the Roche LightCycler®.

Components: Probe-based qRT-PCR ReadyMix
M-MLV Reverse Transcriptase
10× PCR Buffer
25 mM MgCl₂
Reference Dye for Quantitative PCR

Storage: -20 °C

Ordering Information

Cat. No.	Product Description	Quantity
QR0200	qRT-PCR ReadyMix for probe-based applications Sufficient for 100-50 µl reactions	1 kit

Reference Dye for Quantitative PCR

Reference Dye is used for normalization of reaction data when using SYBR Green I dye, Molecular Beacons, or dual-labeled probe chemistries for real time detection. Instrument settings for ROX reference dye are suitable for the measurement of Reference Dye for Quantitative PCR.

0.3 ml solution is sufficient for a minimum of 600 reactions.

Storage: 2-8 °C

Ordering Information

Cat. No.	Product Description	Quantity
R4526	Reference Dye for Quantitative PCR	0.3 ml 5.0 ml

PCR Plate Detection Kit

Perform solid-phase capture and sequence-specific detection of PCR products in an easy-to-use, automatable format, and allele-specific hybridization for the detection and genotyping of point mutations. PCR amplification is carried out with one 5'-biotinylated primer and one unlabeled primer. The amplified products are immobilized in streptavidin-coated strip-well plates. The non-biotinylated strand is removed by sodium hydroxide denaturation, and the biotinylated strand is hybridized to a sequence-specific fluorescein-labeled probe. After a wash step, the probe is detected with a peroxidase-conjugated anti-fluorescein antibody and the chromogenic peroxidase substrate TMB. The assay can be completed in 2.5 hours.

Features and Benefits

- 10-100× more sensitive than standard gel electrophoresis and allows discrimination between PCR product levels over a 2-2.5 log range of concentration. Unique PlateHyb™ hybridization buffer improves genotyping of single base mutations
- Delivers the benefits of antibody inactivated hot start PCR with SYBR Green detection in a ReadyMix specifically designed for capillary instruments; only primers and template are required
- Protocol is easily scalable, allowing processing of as few as eight to several hundred samples. Assay can be completed in 2.5 hours (30-45 minutes hands-on time)

Components: Anti-Fluorescein-Peroxidase Conjugate 500× Stock, 300 µl
Denaturation Solution, 100 ml
DNA Dilution Buffer, 250 ml
PlateHyb Hybridization Buffer, 200 ml
Stop Solution, 100 ml
Streptavidin-Coated 8 × 12 Strip-Well Plates, 5 each
Thermoplate Covers, 15 each
TMB Liquid Substrate System, 2 × 100 ml
Wash Buffer Dry Packs (phosphate buffered saline with Tween 20, pH 7.4), 10 each

Storage: 2-8 °C
Shipped in wet ice

Ordering Information

Cat. No.	Product Description	Quantity
PPD1	PCR Plate Detection Kit Sufficient for 480 detection reactions	1 kit

Protocol for Microplate Detection of Amplicons

