For life science research only.
Not for use in diagnostic procedures.

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LightCycler® RNA Master SYBR Green I

Version: 13
Content version: October 2017

Easy-to-use reaction mix for one-step RT-PCR, using the LightCycler® Carousel-Based System

Cat. No. 03 064 760 001 1 kit
96 reactions of 20 µl final volume each

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial/Bottle	Cap	Label	Function / Description	Content
1	red	LightCycler® RNA Master SYBR Green I, LC RNA Master SYBR Green I, 2.7x conc.	Contains Tth DNA Polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), and SYBR Green I.	3 vials, 250 µl each
2	colorless	LightCycler® RNA Master SYBR Green I, $Mn(OAc)_2$ stock solution, 50 mM	To adjust $Mn(OAc)_2$ concentration in the reaction mix.	1 vial, 1 ml
3	colorless	LightCycler® RNA Master SYBR Green I, Water, PCR Grade	To adjust the final reaction volume.	2 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped on dry ice.
When stored at −15 to −25°C, the kit is stable through the expiration date printed on the label.
Once the kit is opened, store the kit components as described in the following table:

Vial/Bottle	Cap	Label	Storage
1	red	LC RNA Master SYBR Green I, 2.7x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.*** ⚠ ***Keep protected from light.***
2	colorless	$Mn(OAc)_2$ stock solution, 50 mM	Store at −15 to −25°C.
3	colorless	Water, PCR Grade	

1.3. Additional Equipment and Reagents Required

Standard Laboratory Equipment

- Nuclease-free, aerosol-resistant pipette tips
- Sterile reaction tubes for preparing master mixes and dilutions

For RT-PCR

- LightCycler® Carousel-Based System*
- LightCycler® Capillaries*
- Standard benchtop microcentrifuge, containing a rotor for 2.0 ml reaction tubes

ⓘ *The LightCycler® Carousel-Based System includes Centrifuge Adapters that enable LightCycler® Capillaries to be centrifuged in a standard microcentrifuge rotor.*

or

- LC Carousel Centrifuge 2.0* for use with the LightCycler® 2.0 Sample Carousel (20 µl; optional)
- Uracil-DNA Glycosylase, heat-labile* (optional)

ⓘ *For prevention of carryover contamination; see section* **Prevention of Carryover Contamination**.

1.4. Application

LightCycler® RNA Master SYBR Green I is designed for use in research. When combined with the LightCycler® Carousel-Based System, this kit uses a hot start RT-PCR protocol to provide very sensitive detection and quantification of defined RNA sequences (if suitable RT-PCR primers are supplied). The kit is especially suitable for difficult RNA populations, as the elevated incubation temperature during the reverse transcription step will help to overcome secondary structures. The hot start feature will minimize mispriming during the initial phase of the reaction and therefore overall sensitivity of RT-PCR is increased.

In addition, the kit can be used with Uracil-DNA Glycosylase, heat-labile to prevent carryover contamination during PCR.

1.5. Preparation Time

Assay Time

Procedure	Time [min]
RT-PCR Setup	15
Reverse Transcription	20
LightCycler® Carousel-Based System PCR run	25
Total Assay Time	**60**

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- Use any template RNA (e.g., total RNA or mRNA) suitable for RT-PCR in terms of purity, concentration, and absence of inhibitors.
- Use up to 500 ng total RNA or 100 ng mRNA.

⚠ *Using a too high amount of RNA may result in inhibition of the reaction.*

ⓘ *If the concentration of template RNA is lower than 10 µg/ml, the addition of nonspecific carrier RNA (e.g., MS2 RNA*) is recommended to avoid loss of template RNA due to adsorption effects. For optimal results, the total RNA concentration of template plus carrier RNA should not be lower than 10 µg/ml.*

For reproducible isolation of nucleic acids, we recommend:
- Either a MagNA Pure System together with a dedicated reagent kit (for automated isolation),
- or a High Pure Nucleic Acid Isolation Kit (for manual isolation).

Control Reactions

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template RNA with Water, PCR Grade (Vial 3).

DNA Contamination Control

To test the template RNA for contamination with residual genomic DNA, perform PCR of the template RNA in combination with LightCycler® FastStart DNA Master SYBR Green I*, or LightCycler® FastStart DNA Master^{PLUS} SYBR Green I*. As the reverse transcription step is omitted, any PCR product generated is a signal for DNA contamination of the RNA template preparation.

Primers

Suitable concentrations of RT-PCR primers range from 0.2 to 1 µM (final concentration). The recommended starting concentration is 0.5 µM each.

ⓘ *Lower primer concentrations can increase fluorescence intensity at lower target concentrations; higher primer concentrations can increase sensitivity.*

General Considerations

$Mn(OAc)_2$

To ensure specific and efficient amplification with the LightCycler® Carousel-Based System, use $Mn(OAc)_2$ at a final concentration of 3.25 mM.
For most RNA targets tested so far, no titration of $Mn(OAc)_2$ was required. However, if necessary, titrate $Mn(OAc)_2$ in a range from 2.5 to 4 mM, in steps of 0.25 mM. The addition of 0.1 µl 50 mM $Mn(OAc)_2$ stock solution to a final volume of 20 µl, results in an increase of $Mn(OAc)_2$ concentration of 0.25 mM. The volume of water in the RT-PCR reaction must be reduced, accordingly.

2.2. Protocols

Experimental Protocol

LightCycler® Carousel-Based System Protocol

The following procedure is optimized for use with the LightCycler® Carousel-Based System.

⚠ ***Program the LightCycler® Instrument before preparing the reaction mixes.***

A LightCycler® Carousel-Based System protocol that uses the LightCycler® RNA Master SYBR Green I contains the following programs:

- **Reverse Transcription** of template RNA
- **Pre-Incubation** for denaturation of cDNA/RNA hybrid
- **Amplification** of the cDNA
- **Melting Curve** for PCR product identification/amplicon analysis
- **Cooling** the rotor and the thermal chamber

ⓘ *For details on how to program the experimental protocol, see the LightCycler® Operator's Manual.*

⚠ ***Set all other protocol parameters not listed in the table below to 0.***

The following table shows the RT-PCR parameters that must be programmed for a LightCycler® Carousel-Based System RT-PCR run with the LightCycler® RNA Master SYBR Green I.

LightCycler® Software Version 4.1		
Programs		
Setup	Setting	
Default Channel	Fluorescence Channel 530	
Seek Temperature	61°C	
Max Seek Pos.	Enter the total number of sample positions the instrument should look for.	
Instrument Type	"6 Ch." for LightCycler® 2.0 Instrument or "3 Ch." for LightCycler® 1.5 Instrument	
Capillary Size	Select "20 µl" as the capillary size for the experiment. Available only for LightCycler® 2.0 Instrument (6 channels).	
Programs		
Program Name	Cycles	Analysis Mode
Reverse Transcription	1	None
Pre-Incubation	1	None
Amplification	45	Quantification
Melting Curve	1	Melting Curves
Cooling	1	None

LightCycler® Software Version 4.1				
Programs				
Temperature Targets				
	Target [°C]	Hold [hh:mm:ss]	Ramp Rate[1] [°C/s]	Acquisition Mode [per °C]
Reverse Transcription	61	00:20:00[2]	20	None
Pre-Incubation	95	00:00:30[3]	20	None
Amplification	95	00:00:01 – 00:00:05[3]	20	None
	primer dependent[4]	00:00:05 – 00:00:20[5]	20	None
	72[6]	00:00:05 – 00:00:30[5,7]	20	Single
Melting Curve	95	00:00:00	20	None
	65	00:00:15	20	None
	95	00:00:00	0.1	Continuous
Cooling	40	00:00:30	20	None

[1] Temperature Transition Rate/Ramp Rate is 20°C/second, except where indicated.

[2] When amplifying GC-rich templates, or templates with a high degree of secondary structures, it is recommended to extend the reverse transcription incubation time to 30 minutes or longer.

[3] When amplifying GC-rich templates or templates with a high degree of secondary structure, it is recommended to increase the Initial Denaturation incubation time up to 2 minutes and the Denaturation time in the program “Amplification” up to 5 seconds.

[4] For initial experiments, set the target temperature (i.e., the primer annealing temperature) 5°C below the calculated primer Tm. Calculate the primer Tm according to the following formula, based on the nucleotide content of the primer:
Tm = 2°C (A + T) + 4°C (G + C).

[5] For greater precision in target quantification experiments, it can be advantageous in some cases to choose longer annealing and extension times for the amplification cycles.

[6] If the primer annealing temperature is low (< +55°C), reduce the Ramp Rate to 2 to 5°C/second.

[7] Calculate the hold time for the PCR elongation step by dividing the amplicon length over 25 (e.g., a 500 bp amplicon requires 20 seconds elongation time).

Preparation of the RT-PCR Mix

Proceed as described below for a 20 µl standard reaction.

⚠ ***Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.***

1. Depending on the total number of reactions, place the required number of LightCycler® Capillaries into pre-cooled centrifuge adapters, or into a LightCycler® Sample Carousel in a pre-cooled LC Carousel Centrifuge Bucket.

2. Thaw the solutions, and for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening.
   – Mix carefully by pipetting up and down and store on ice.

3. Prepare a 10x conc. solution of RT-PCR primers.

   If you are using the recommended final concentration of 0.5 µM for each primer, the 10x conc. solution would contain a 5 µM concentration of each primer.

4. In a 1.5 ml reaction tube on ice, prepare the RT-PCR Mix for one 20 µl reaction by adding the following components in the order mentioned below:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade (Vial 3)	8.2	–
$Mn(OAc)_2$ stock solution, 50 mM (Vial 2)	1.3	3.25 mM
RT-PCR Primer Mix, 10x conc.[1]	2.0	0.2 to 1.0 µM each (recommended concentration is 0.5 µm)
LightCycler® RNA Master SYBR Green I (Vial 1)	7.5	1x
Total Volume	**19**	

   To prepare the RT-PCR Mix for more than one reaction, multiply the amount in the "Volume" column above by z, where z = the number of reactions to be run plus one additional reaction.

5. Mix gently by pipetting up and down. Do not vortex.
   – Pipette 19 µl RT-PCR mix into each pre-cooled LightCycler® Capillary.
   – Add 1 µl of the RNA template.
   – Seal each capillary with a stopper.

6. Place the centrifuge adapters containing the capillaries into a standard benchtop microcentrifuge. Place the centrifuge adapters in a balanced arrangement within the centrifuge.
   – Centrifuge at 700 × *g* for 5 seconds (3,000 rpm) in a standard benchtop microcentrifuge.
   – Alternatively, use the LC Carousel Centrifuge for spinning the capillaries.

7. Transfer the capillaries into the LightCycler® Sample Carousel and then into the LightCycler® Instrument.

8. Cycle the samples, as described above.

[1] Due to possible primer/primer interactions generated during storage, it may be necessary to preheat the RT-PCR primer mix for 1 minute at 95°C, before starting the reaction to achieve optimal sensitivity.

2.3. Other Parameters

Prevention of Carryover Contamination

Uracil-DNA Glycosylase, heat-labile (UNG, heat-labile) is suitable for preventing carryover contamination in PCR. This carryover prevention technique involves incorporating deoxyuridine triphosphate (dUTP, a component of the reaction mixes of all LightCycler® reagent kits) into amplification products, then pretreating later PCR mixtures with UNG. If a dUTP-containing contaminant is present in the later PCRs, it will be cleaved by a combination of the UNG and the high temperatures of the initial denaturation step; it will not serve as a PCR template.
Proceed as described below to prevent carryover contamination using UNG, heat-labile:

1. Add 1 µl UNG, heat-labile to the master mix per 20 µl final reaction volume.

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2. Add template RNA and incubate the completed reaction mixture for 5 minutes at +15 to +25°C.

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3. Destroy any contaminating template and inactivate the UNG enzyme by performing the reverse transcription step at +61°C.

   ⚠ ***Do not perform an additional inactivation step at higher temperatures than 61°C, as the reverse transcriptase would be inactivated.***

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i *When performing Melting Curve analysis, the use of UNG may lower the melting temperature (Tm) by approximately 1°C.*

3. Results

Quantification Analysis

The following amplification curves were obtained using the LightCycler® RNA Master SYBR Green I, in combination with the LightCycler® Control Kit RNA, targeting *in vitro*-transcribed cytokine RNA template. The fluorescence values versus cycle number are displayed. Fifty copies of the cytokine RNA can be reproducibly detected by amplification in the LightCycler® Carousel-Based System and using the SYBR Green I detection format.
For high amounts of template RNA, the maximal fluorescence signal using SYBR Green I dye as a detection format may be reduced due to dye limitations.

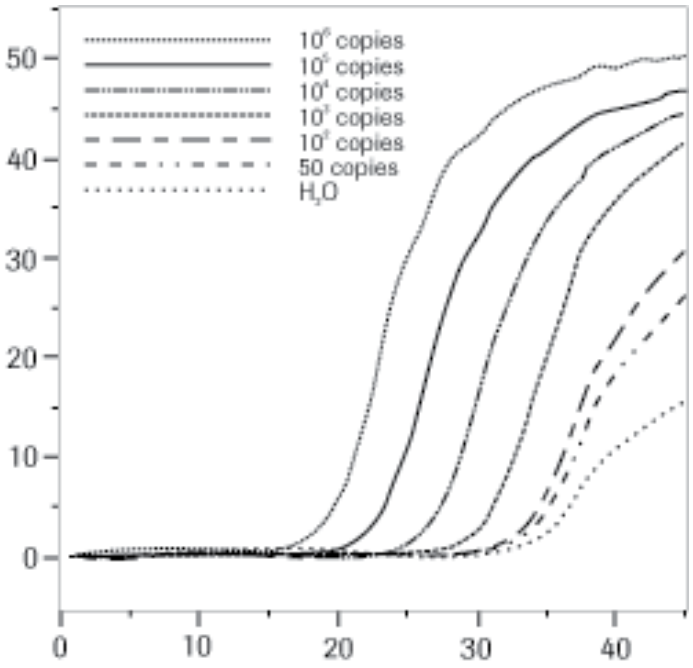

Fig. 1: Serially diluted samples containing 50 copies (far right) to 10^6 copies (far left) of cytokine RNA template from the LightCycler® Control Kit RNA were amplified using the LightCycler® RNA Master SYBR Green I in a LightCycler® Carousel-Based System Instrument. As a negative control, template RNA was replaced by Water, PCR Grade (flat line).

Melting Curve Analysis

Specificity of the amplified PCR product was assessed by performing a Melting Curve analysis. The resulting melting curves enable discrimination between primer-dimers and specific PCR product. The specific cytokine product melts at a higher temperature than the primer-dimers. The melting curves display the specific amplification of the cytokine PCR product. In the negative control sample, only primer-dimers are seen.

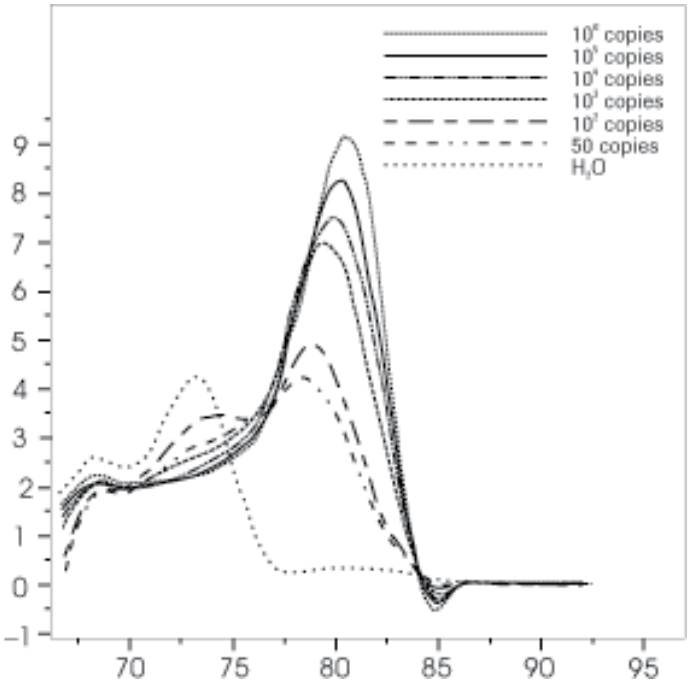

Fig. 2: Melting Curve analysis of amplified samples containing 50 copies (lowest peak) to 10^6 copies (highest peak) of cytokine RNA. As a negative control, the template RNA was replaced by Water, PCR Grade (flat line).

4. Troubleshooting

Observation	Possible cause	Recommendation
Amplification reaches plateau phase before the program is complete.	Very high starting amount of nucleic acid.	The program can be finished by clicking on the **End Program** button. The next cycle program will start automatically.
	The number of cycles is too high.	Reduce the number of cycles in the amplification program.
Log-linear phase of amplification just starts as the amplification program finishes.	Very low starting amount of nucleic acid.	Increase the number of cycles by 10 in the amplification program.
		Improve PCR conditions (*e.g.,* primer concentration or design).
		Use higher amount of starting material.
		Repeat the run.
	The number of cycles is too low.	Increase the number of cycles in the amplification program.
		Use the **+10 cycles** button to increase the number of cycles in the amplification program.
No amplification occurs.	Wrong channel has been chosen to display amplification online.	Change the channel settings on the programming screen. The data obtained up to this point will be saved.
	Scale of axes on graph are unsuitable for analysis.	Change the values for the x- and y-axis by double-clicking on the maximum and/or minimum values, then change to more suitable values.
	Measurements do not occur.	Check the amplification program. For SYBR Green I detection format, choose “single” as acquisition mode at the end of the elongation phase.
	Pipetting errors or omitted reagents.	Check for missing reagents.
		Check for defective SYBR Green I dye.
		Check experimental protocol.
		Always run a positive control along with the samples.
		Increase amount of RNA template up to 1 µg total RNA or mRNA.
	Inappropriate $Mn(OAc)_2$ concentration.	Titrate $Mn(OAc)_2$ in a range of 2.5 to 4 mM.
	Poor PCR efficiency due to unsuitable primers.	Check RT-PCR product on an agarose gel.
		Redesign primers.
		Check annealing temperature of primers.
		Primer concentration should be in the range of 0.2 to 1.0 µM.
Fluorescence intensity is too low.	Low concentration or deterioration of SYBR Green I dye in the reaction mixtures due to unsuitable storage conditions.	Store the SYBR Green I dye containing reagents at −15 to −25°C and keep protected from light.
		Avoid repeated freezing and thawing.
Fluorescence intensity varies.	RT-PCR is still in the upper part of the capillary. Air bubble is trapped in the capillary tip.	Repeat capillary centrifugation step.
	Skin oils on the surface of the capillary tip.	Always wear gloves when handling the capillaries.

Observation	Possible cause	Recommendation
Poor PCR efficiency.	Reaction conditions are not optimized leading to poor PCR efficiency.	Primer concentration should be in the range of 0.2 to 1.0 µM.
		Check annealing temperature of the primers.
		Check experimental protocol.
		Always run a positive control along with the samples.
		Increase amount of RNA template up to 500 ng total RNA or 100 ng mRNA.
		For most RNA targets tested so far, no titration of $Mn(OAc)_2$ has been required. However, if necessary, a titration of $Mn(OAc)_2$ in a range from 2.5 to 4 mM in steps of 0.25 mM may be advantageous (0.1 µl $Mn(OAc)_2$ stock solution, 50 mM corresponds to 0.25 mM $Mn(OAc)_2$ in a final volume of 20 µl).
	Amplicon length is larger than 750 bp.	Do not use amplicons larger than 750 bp. Amplification efficiency is reduced with amplicons of this size. Optimal results are obtained with amplicons up to 500 bp.
	Inhibitory effects of the sample material due to insufficient purification.	Do not use more than 7 to 8 µl of RNA per 20 µl RT-PCR reaction mixture.
		Dilute sample 1:10 and repeat the analysis.
		Repurify the nucleic acids to ensure removal of inhibitory agents.
	RNA degradation due to improper storage or isolation.	Check RNA quality on a gel.
		Check RNA with an established primer pair, if available.
Amplification curve reaches plateau at a lower signal level than the other samples.	Starting amount of RNA is too high; RNA captures SYBR Green I dye leading to a high background signal. Insufficient amounts of SYBR Green I dye are left to monitor the increase of the fluorescence signal during the amplification.	Instead of SYBR Green I, use the HybProbe format which enables analysis of up to 1 µg RNA.
	SYBR Green I dye bleached.	Ensure that the reagents containing the SYBR Green I dye are stored protected from light. Avoid repeated freezing and thawing.
Negative control samples are positive.	Contamination	Exchange all critical solutions.
		Pipette reagents on a clean bench.
		Close lid of the negative control reaction immediately after pipetting.
		Use UNG, heat-labile for prevention of carryover contamination.
Melting peak is very broad and peaks cannot be differentiated.	**°C to Average** setting is too high.	Reduce the value of **°C to Average** (only applicable for LightCycler® Software versions prior to version 4.0).
Double melting peak appears for one product.	Two products of different length or GC-content have been amplified (*i.e.,* pseudogenes or mispriming).	Check products on an agarose gel.
		Elevate the stringency by: ▪ redesigning the primers, ▪ checking the annealing temperature, ▪ performing a “touch-down” PCR, or ▪ using HybProbe probes for better specificity.

Observation	Possible cause	Recommendation
Only a primer-dimer peak appears, with no specific PCR product peak, or very high primer-dimer peaks.	Primer-dimers have outcompeted amplification of specific PCR product.	Keep all samples at +2 to +8°C on ice until the run is started.
		Keep the time between preparing the reaction mixture and starting the run as short as possible.
		See also recommendations under “Poor PCR efficiency”.
Primer-dimer and product peaks are very close together.	Unusually high GC-content of the primers.	Redesign primers.
		Run melting curve at the lowest ramp rate (0.1°C/second and continuous measurement).
		Spread scale of the x-axis.
		Lower the number of **°C to Average** (only applicable for LightCycler® Software versions prior to version 4.0).
Tm shift of product melting peak up to 2°C.	High amount of sample material results in higher Tm of the melting peak than low target concentrations.	This has no influence on quantification results. Under the reaction conditions used in this kit, higher concentrations of PCR products result in a slight increase of Tm compared to less PCR products.
Very broad primer-dimer peak with multiple peaks.	Heterogeneous primers with primer-dimer variations (*e.g.,* concatemers, loops).	Redesign primers.
One peak of the same height occurs in all samples.	Contamination in all samples.	Close capillaries during centrifugation step.
		Use fresh solutions.

5. Additional Information on this Product

5.1. Test Principle

The hot start feature of the LightCycler® RNA Master SYBR Green I is achieved by using Tth DNA Polymerase, in combination with aptamers. Tth DNA Polymerase is a thermostable enzyme with RNA-dependent reverse transcriptase activity and DNA-dependent polymerase activity, allowing the combination of RT and PCR in a single-tube reaction. Aptamers are dedicated oligonucleotides that bind in the active center of the polymerase and prevent attachment to nucleic acid targets at temperatures below the optimal reaction temperature of the Tth enzyme. Therefore, no primer elongation occurs during setup of the reaction and the following heating phase prior to the RT step. At higher temperatures, the aptamers are released from the enzyme and RT or DNA polymerization can be initiated. In addition, the recommended incubation temperature for reverse transcription with Tth (61°C) is helpful to overcome secondary structures of RNA. This results in highly specific and efficient cDNA synthesis that leads to highly specific and sensitive PCR. Hot start with aptamers is highly effective and very convenient, because it does not require additional incubation steps, pipetting steps, or an extension of reaction time. The hot start protocol with aptamers does not interfere with other enzymatic processes, the online detection of amplification products, or subsequent handling steps.

Generation of PCR products can be detected by measurement of the SYBR Green I fluorescence signal. SYBR Green I intercalates into the DNA double helix. In solution, the unbound SYBR Green I dye exhibits very little fluorescence; however, fluorescence (530 nm) is greatly enhanced upon DNA binding. Therefore during PCR, the increase in SYBR Green I fluorescence is directly proportional to the amount of double-stranded DNA generated.
As SYBR Green I dye is very stable (only 6% of the activity is lost during 30 amplification cycles) and the LightCycler® Instruments' optical filter set matches the wavelengths of excitation and emission, it is the reagent of choice when measuring total DNA.

The basic steps of DNA detection by SYBR Green I during real-time PCR on the LightCycler® Carousel-Based System are:

① At the beginning of amplification, the reaction mixture contains the denatured DNA, the primers, and the SYBR Green I dye. The unbound SYBR Green I dye molecules weakly fluoresce, producing a minimal background fluorescence signal, which is subtracted during computer analysis.

② After annealing of the primers, a few SYBR Green I dye molecules can bind to the double strand. DNA binding results in a dramatic increase of the SYBR Green I dye molecules to emit light upon excitation.

③ During elongation, more and more SYBR Green I dye molecules bind to the newly synthesized DNA. If the reaction is monitored continuously, an increase in fluorescence is viewed in real-time. Upon denaturation of the DNA for the next heating cycle, the SYBR Green I dye molecules are released and the fluorescence signal falls.

④ Fluorescence measurement at the end of the elongation step of every PCR cycle is performed to monitor the increasing amount of amplified DNA.

To prove that only the desired PCR product has been amplified, perform a Melting Curve analysis after PCR. In Melting Curve analysis, the reaction mixture is slowly heated to +95°C, which causes melting of double-stranded DNA and a corresponding decrease of SYBR Green I fluorescence. The Instrument continuously monitors this fluorescence decrease and displays it as melting peaks. Each melting peak represents the characteristic melting temperature (Tm) of a particular DNA product (where the DNA is 50% double-stranded and 50% single-stranded). The most important factors that determine the Tm of dsDNA are the length and the GC-content of that fragment. If PCR generated only one amplicon, Melting Curve analysis will show only one melting peak. If primer-dimers or other nonspecific products are present, they will be shown as additional melting peaks. Checking the Tm of a PCR product can thus be compared with analyzing a PCR product by length in gel electrophoresis.

How this Product Works

LightCycler® RNA Master SYBR Green I is an easy-to-use hot start reaction mix, specifically designed for one-step RT-PCR in 20 µl glass capillaries using the LightCycler® Carousel-Based System and SYBR Green I as the detection format. Amplification and online monitoring of the template RNA is achieved by a combined procedure on the LightCycler® Carousel-Based System Instrument. The results are interpreted directly after completing the PCR and Melting Curve. The amplicon is detected by measurement of the SYBR Green I fluorescence signal.
The LightCycler® RNA Master SYBR Green I provides convenience, high performance, reproducibility, and minimizes contamination risk. All you need to supply is template RNA and RT-PCR primers.

In principle, the LightCycler® RNA Master SYBR Green I can be used for the amplification and detection of any RNA target. However, the amplification protocol must be optimized to the reaction conditions of the LightCycler® Carousel-Based System and specific RT-PCR primers designed for each target. Refer to the LightCycler® Operator's Manual for general recommendations.

⚠ ***The amplicon size should not exceed 750 bp in length. For optimal results, select a product length of 500 bp or less.***
⚠ ***The performance of the kit described in this Instructions for Use is guaranteed only when it is used with the LightCycler® Carousel-Based System.***

5.2. Quality Control

The LightCycler® RNA Master SYBR Green I is function tested with the LightCycler® Control Kit RNA, using the LightCycler® Carousel-Based System.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Accessories general (hardware)		
LightCycler® Software 4.1	1 software package	04 898 915 001
LC Carousel Centrifuge 2.0	1 centrifuge plus rotor and rotor bucket (115 V)	03 709 507 001
	1 centrifuge plus rotor and rotor bucket (230 V)	03 709 582 001
Consumables		
LightCycler® Capillaries (20 µl)	5 x 96 capillaries, containing 5 boxes, each with 96 capillaries and stoppers, 5 boxes, each with 96 capillaries and stoppers	04 929 292 001
Instruments		
LightCycler® 2.0 Instrument	1 instrument	03 531 414 001
Reagents , kits		
LightCycler® FastStart DNA Master SYBR Green I	1 kit, 96 reactions of 20 µl final volume each	03 003 230 001
	1 kit, 480 reactions of 20 µl final volume each	12 239 264 001
LightCycler® FastStart DNA Master^{PLUS} SYBR Green I	1 kit, 96 reactions of 20 µl final volume each	03 515 869 001
	1 kit, 480 reactions of 20 µl final volume each	03 515 885 001
	1 kit, 1,920 reactions of 20 µl or 384 reactions of 100 µl final volume each	03 752 186 001
Uracil-DNA Glycosylase, heat-labile	100 U, 1 U/µl	11 775 367 001
	500 U, 1 U/µl	11 775 375 001
RNA, MS2	500 µl, 10 A260 units	10 165 948 001

6.4. Trademarks

FASTSTART, HIGH PURE, HYBPROBE, LIGHTCYCLER and MAGNA PURE are trademarks of Roche.
SYBR is a trademark of Thermo Fisher Scientific Inc..
All third party product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to: http://technical-support.roche.com.

6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

Visit **lifescience.roche.com**, to download or request copies of the following **Materials**:
- Instructions for Use
- Safety Data Sheets
- Certificates of Analysis
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