

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



# LightCycler<sup>®</sup> RNA Amplification Kit SYBR Green I

 **Version: 15**

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Kit for One-Step RT-PCR using the LightCycler<sup>®</sup> Carousel-Based System

**Cat. No. 12 015 137 001**    1 kit  
96 reactions of 20 µl final volume each

**Store the kit at –15 to –25°C.**

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# 1. General Information

## 1.1. Contents

Vial/Bottle	Cap	Label	Function/Description	Content
1	green	LightCycler® RNA Amplification Kit SYBR Green I, LC RT-PCR Enzyme Mix	Enzyme mix for RT-PCR.	2 vials, 20 µl each
2	green	LightCycler® RNA Amplification Kit SYBR Green I, LC RT-PCR Reaction Mix, SYBR Green I, 5x conc.	<ul style="list-style-type: none"> <li>Reaction mix for RT-PCR.</li> <li>Contains reaction buffer, dNTP mix (with dUTP instead of dTTP), SYBR Green I dye, and 15 mM MgCl<sub>2</sub>.</li> </ul>	3 vials, 128 µl each
3	blue	LightCycler® RNA Amplification Kit SYBR Green I, MgCl <sub>2</sub> stock solution, 25 mM	To adjust MgCl <sub>2</sub> concentration.	1 vial, 1 ml
4	colorless	LightCycler® RNA Amplification Kit SYBR Green I, Water, PCR Grade	To adjust the final reaction volume.	2 vials, 1 ml each
5	colorless	LightCycler® RNA Amplification Kit SYBR Green I, Resolution solution, 5x conc.	To amplify RNA templates of high GC-content or high degree of secondary structures.	1 vial, 1 ml

## 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	green	LC RT-PCR Enzyme Mix	Store at –15 to –25°C.
2	green	LC RT-PCR Reaction Mix, SYBR Green I, 5x conc.	<b>⚠ Avoid repeated freezing and thawing.</b> <b>⚠ Aliquot Reaction Mix (Vial 2) and keep protected from light.</b>
3	blue	MgCl <sub>2</sub> stock solution, 25 mM	
4	colorless	Water, PCR Grade	Store at –15 to –25°C.
5	colorless	Resolution solution, 5x conc.	

### 1.3. Additional Equipment and Reagent 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

The LightCycler® RNA Amplification Kit SYBR Green I is designed for use in life science research. The kit provides reagents, including RT-PCR enzyme mix, reaction mix, MgCl<sub>2</sub>, Resolution Solution, and Water, PCR Grade for one-step RT-PCR in glass capillaries using the LightCycler® Carousel-Based System and SYBR Green I as detection format. 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	Assay Time [min]
PCR Setup	15
Reverse Transcription	10
LightCycler® Carousel-Based System PCR run	25
<b>Total Assay Time</b>	<b>50</b>

## 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.**

**i** *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 4).

##### 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<sup>PLUS</sup> 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 PCR primers range from 0.2 to 1 µM (final concentration). The recommended starting concentration is 0.5 µM each.

##### Mg<sup>2+</sup> Concentration

To ensure specific and efficient amplification with the LightCycler® Carousel-Based System, you must optimize the MgCl<sub>2</sub> concentration for each target. The LightCycler® RT-PCR Reaction Mix SYBR Green I contains a MgCl<sub>2</sub> concentration of 3 mM (final concentration). The optimal concentration for RT-PCR with the LightCycler® Carousel-Based System may vary from 3 to 7 mM.

The table below gives the volumes of the MgCl<sub>2</sub> stock solution (Vial 3) that you must add to a 20 µl reaction (final RT-PCR volume) to increase the MgCl<sub>2</sub> concentration to the indicated values.

To reach a final Mg <sup>2+</sup> concentration [mM] of:	3	4	5	6	7
Add this amount of 25 mM MgCl <sub>2</sub> stock solution [µl]	0	0.8	1.6	2.4	3.2

**i** *The volume of water in the RT-PCR reaction must be reduced, accordingly.*

### 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.
- 2 Add template RNA and incubate the completed reaction mixture for 5 minutes at +15 to +25°C.
- 3 Destroy any contaminating template and inactivate the UNG enzyme by performing the reverse transcription step at +55°C.

**⚠ Do not perform an additional inactivation step at higher temperatures (+55°C), as the reverse transcriptase would be inactivated.**

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

### Safety Information

#### For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

## 2.2. Protocols

### 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 Amplification Kit 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

**i** For details on how to program the 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 Amplification Kit SYBR Green I.

LightCycler® Software Version 4.1				
Programs				
Setup	Setting			
Default Channel	Fluorescence Channel 530			
Seek Temperature	55°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 Curve		
Cooling	1	None		
Temperature Targets				
	Target [°C]	Hold [hh:mm:ss]	Ramp Rate <sup>(1)</sup> [°C/s]	Acquisition Mode [per °C]
Reverse Transcription	55	00:10:00 <sup>(2)</sup>	20	None
Pre-Incubation	95	00:00:30	20	None
Amplification	95	00:00:00 <sup>(3)</sup>	20	None
	primer dependent <sup>(4)</sup>	00:00:10	20	None
	72 <sup>(5)</sup>	00:00:05 - 00:00:30 <sup>(6,7)</sup>	20	Single
Melting Curve	95	00:00:00	20	None
	65	00:00:10	20	None
	95	00:00:00	0.1	Continuous
Cooling	40	00:00:30	20	None

<sup>(1)</sup> Temperature Transition Rate/Slope is 20°C/second, except where indicated.

<sup>(2)</sup> 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.

<sup>(3)</sup> When amplifying GC-rich templates or templates with a high degree of secondary structures, it is recommended to extend the denaturation incubation time to 5 seconds.

<sup>(4)</sup> For initial experiments, set the target temperature (*i.e.*, the primer annealing temperature) 5°C below the calculated primer T<sub>m</sub>. Calculate the primer T<sub>m</sub> according to the following formula, based on the nucleotide content of the primer:  
T<sub>m</sub> = 2°C (A + T) + 4°C (G + C).

<sup>(5)</sup> If the primer annealing temperature is low (< +55°C), reduce the temperature transition rate/slope to 2 to 5°C/second.

<sup>(6)</sup> 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.

<sup>(7)</sup> 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).

## 2. How to Use this Product

### 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 PCR primers.
  - i* 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 4)	X <sup>(1)</sup>	-
LC RT-PCR Reaction Mix SYBR Green I, 5x conc. (Vial 2)	4.0	1x
Resolution Solution, 5x conc. (Vial 5)	Y <sup>(1)</sup>	0.5 - 1x
MgCl <sub>2</sub> stock solution (Vial 3)	Z	Use concentration that is optimal for the target.
Primer Mix <sup>(2)</sup> , 10x conc.	2.0	0.2 to 1.0 µM each (recommended conc. is 0.5 µM)
LC RT-PCR Enzyme Mix (Vial 1)	0.4	-
<b>Total Volume</b>	<b>19</b>	

*i* To prepare the RT-PCR Mix for more than one reaction, multiply the amount in the "Volume" column above by 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.

<sup>(1)</sup> When amplifying GC-rich templates or templates with a high degree of secondary structure, use 2 to 4 µl Resolution Solution per 20 µl standard reaction. Adjust the volume of water accordingly.

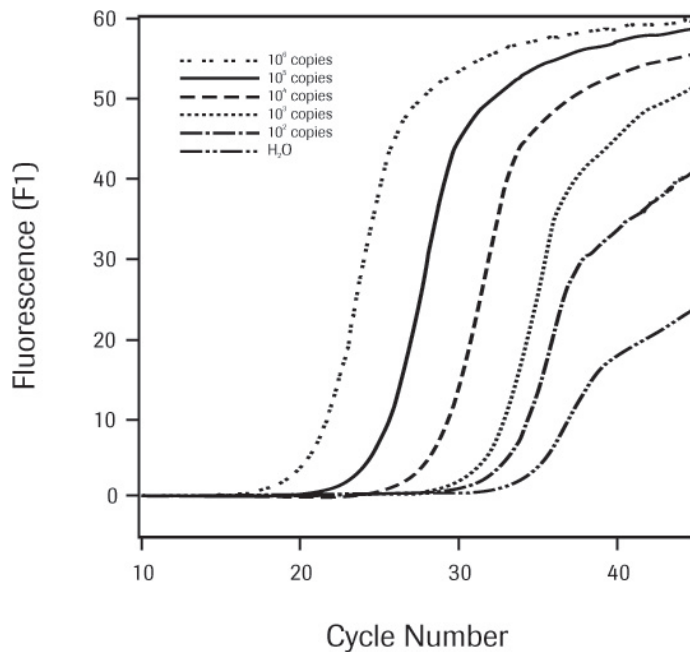
<sup>(2)</sup> Due to possible primer/primer interactions generated during storage, it may be necessary to preheat the PCR primer mix for 1 minute at +95°C before starting the reaction to achieve optimal sensitivity.



### 3. Results

#### Quantification Analysis

The following amplification curves were obtained using the LightCycler® RNA Amplification Kit 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. One-hundred copies of the cytokine RNA can be reproducibly detected by amplifying in the LightCycler® Carousel-Based System and using the SYBR Green I detection format.

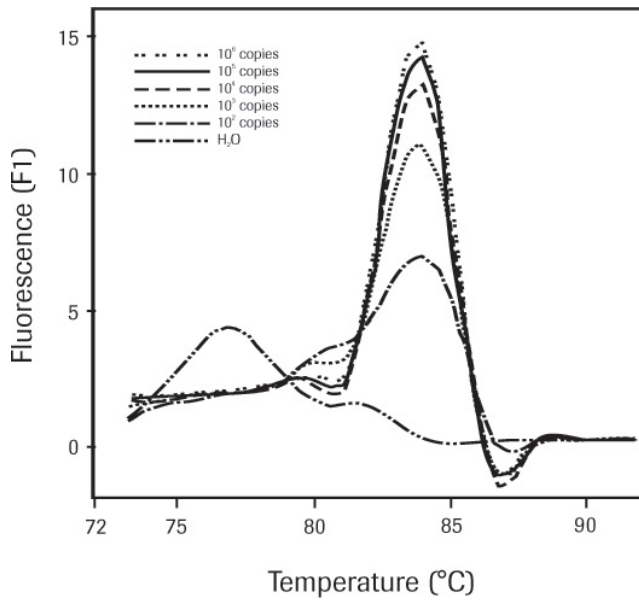


**Fig. 1:** Serially diluted samples containing 10<sup>2</sup> (far right) to 10<sup>6</sup> (far left) copies of cytokine RNA template from the LightCycler® Control Kit RNA were amplified using the LightCycler® RNA Amplification Kit SYBR Green I in a LightCycler® Carousel-Based System Instrument. As a negative control, template RNA was replaced by Water, PCR Grade (flat line).

### 3. Results

#### 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 were amplified.



**Fig. 2:** Melting Curve analysis of amplified samples containing 10<sup>2</sup> (lowest peak) to 10<sup>6</sup> (highest peak) copies of cytokine RNA. As a negative control, the template RNA was replaced by Water, PCR Grade.

## 4. Troubleshooting

Observation	Possible cause	Recommendation
Precipitate in RT-PCR reaction buffer.	Concentrated compounds in the RT-PCR reaction buffer, in combination with storage conditions.	Place the RT-PCR reaction mix at +15 to +25°C. Mix gently until the precipitate is completely dissolved and place on ice.
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 <b>End Program</b> 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 ( <i>e.g.</i> , MgCl <sub>2</sub> concentration, primer concentration or design).
		Use higher amount of starting material.
	Repeat the run.	
	Number of cycles is too low.	Increase the number of cycles in the amplification program.

## 4. Troubleshooting

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.
	Pipetting errors or omitted reagents.	Check for missing reagents.
		Titrate MgCl <sub>2</sub> concentration.
		Check for defective SYBR Green I dye.
		Check experimental protocol.
		Always run a positive control along with your samples.
	Increase amount of RNA template up to 1 mg total RNA or mRNA.	
	Chosen gain settings are too low.	<b>⚠️ Optimize gain settings using the Real Time Fluorimeter function, then repeat the run using the optimal gain settings in the cycle programs.</b>
	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.	
Poor PCR efficiency due to unsuitable primers.	Check PCR product on an agarose gel.	
	Redesign primers.	
	Check annealing temperature of primers.	
Amplicon length is >1 kb.	Primer concentration should be in the range of 0.2 to 1.0 μM.	
	Do not use amplicons >1 kb. Amplification efficiency is reduced with amplicons of this size. Optimal results are obtained for amplicons of 700 bp or less.	
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.	
	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.	
Fluorescence intensity is too high and reaches overflow.	Unsuitable gain settings.	Gain settings cannot be changed during or after a run. Before repeating the run, use the <b>Real Time Fluorimeter</b> option to find suitable gain settings. For SYBR Green I, the background fluorescence at measuring temperature should not exceed 10. <b>⚠️ Use an extra sample for this procedure so that the dyes in your experimental samples will not be bleached. LightCycler® Software versions 3.5 and higher do not require a gain setting.</b>

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.
	Chosen gain settings are too low.	<b>⚠ Optimize gain settings using the Real Time Fluorimeter function, then repeat the run using the optimal gain settings in the cycle programs.</b>
	Reaction conditions are not optimized leading to poor PCR efficiency.	Titrate MgCl <sub>2</sub> concentration. Primer concentration should be in the range of 0.2 to 1.0 µM. Check annealing temperature of primers. Check experimental protocol. Always run a positive control along with your samples. Increase amount of RNA template up to 500 ng total RNA or 100 ng mRNA.
Fluorescence intensity varies.	Poor PCR efficiency due to high GC-content, or high degree of secondary structures of the RNA.	Extend the incubation time for Reverse Transcription to 30 minutes and for denaturation during cycling, to 5 seconds.
	PCR mix is still in the upper part of the capillary. Air bubble is trapped in the capillary tip.	Repeat capillary centrifugation step.
Amplification curve reaches plateau at a lower signal level than the other samples.	Skin oils on the surface of the capillary tip.	Always wear gloves when handling the capillaries.
	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 fluorescence signal during amplification.	Instead of SYBR Green I, use the HybProbe format which enables analysis of up to 1 µg RNA.
Negative control samples are positive.	SYBR Green I dye bleached.	Ensure the reagents containing the SYBR Green I dye are stored protected from light. Avoid repeated freezing and thawing.
	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 <b>°C to Average</b> (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>i.e.</i> , pseudogenes or mispriming).	Check products on an agarose gel. Elevate the reaction stringency by: <ul style="list-style-type: none"> <li>▪ redesigning the primers,</li> <li>▪ checking the annealing temperature,</li> <li>▪ performing a “touch-down” PCR, or</li> <li>▪ using HybProbe probes for better specificity.</li> </ul>
Melting temperature of a product varies from experiment to experiment.	Variations in reaction mixture ( <i>e.g.</i> , salt concentration).	Check purity of template solution. Reduce variations in parameters such as MgCl <sub>2</sub> , heat-labile UNG, and program parameters.

## 4. Troubleshooting

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”.
	Quality of primers is poor.	Use a higher grade primer purification or purify primers more thoroughly.
	Sequence of primers is inappropriate.	Redesign primers.
Primer-dimer and product peaks are very close together.	Unusually high GC-content of the primers.	Redesign primers.
		Run melting curve at the lowest ramping rate (0.1°C/second and continuous measurement).
		Expand scale of the x-axis. Reduce the value of <b>°C to Average</b> (only applicable for LightCycler® Software versions prior to version 4.0).
Very broad primer-dimer peak with multiple peaks.	Heterogeneous primers with primer-dimer variations ( <i>e.g.</i> , 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

Generation of PCR products can be detected by measurement of the SYBR Green I fluorescence signal. SYBR Green I dye (already included in the Reaction Mix of the LightCycler® RNA Amplification Kit SYBR Green I) will emit a fluorescence signal (530 nm) only when intercalated into the DNA double helix. Therefore during PCR, the increase in SYBR Green I fluorescence is directly proportional to the amount of double-stranded DNA generated.

Specificity and sensitivity of amplification reactions detected with SYBR Green I dye is greatly enhanced by combining amplification with a Melting Curve analysis. 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 of a particular DNA product (where the DNA is 50% double-stranded and 50% single-stranded). 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.

### How this Product Works

The LightCycler® RNA Amplification Kit SYBR Green I is designed specifically for the SYBR Green I detection format using the LightCycler® Carousel-Based System. It is used to perform one-step RT-PCR in 20 µl glass capillaries. Amplification and online monitoring of the template RNA is achieved by a combined procedure on the LightCycler® Carousel-Based System Instruments. The results are interpreted directly after completing the PCR. The amplicon is detected by measurement of the SYBR Green I fluorescence signal.

The LightCycler® RNA Amplification Kit SYBR Green I provides convenience, high performance, reproducibility, and minimizes contamination risk. All you need to supply is template RNA, PCR primers, and additional MgCl<sub>2</sub> (if necessary).

In principle, the LightCycler® RNA Amplification Kit SYBR Green I can be used for the amplification and detection of every RNA target. However, you would need to optimize each amplification protocol to the reaction conditions of the LightCycler® Carousel-Based System.

**⚠ The amplicon size should not exceed 1 kb in length. For optimal results, select a product length of 700 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 Amplification Kit 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.	
   etc.	Stages in a process that usually occur in the order listed.
   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

New information added related to the REACH Annex XIV

### 6.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a full overview of related products and manuals, please visit and bookmark our homepage [lifescience.roche.com](http://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)	12 189 682 001
	1 centrifuge plus rotor and rotor bucket (230 V), <i>Not available in US</i>	03 709 507 001
		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
Reagents, kits		
RNA, MS2	500 µl, 10 A <sub>260</sub> units	10 165 948 001
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 <sup>PLUS</sup> 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
Uracil-DNA Glycosylase, heat-labile	1 kit, 1,920 reactions of 20 µl or 384 reactions of 100 µl final volume each	03 752 186 001
	100 U, 1 U/µl	11 775 367 001
	500 U, 1 U/µl	11 775 375 001



## 6.4. Trademarks

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## 6.5. License Disclaimer

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## 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.

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