For general laboratory use.
FOR *IN VITRO* USE ONLY.

Roche Applied Science

LightCycler® RNA Amplification Kit SYBR Green I

Version December 2004

Kit for One-Step RT-PCR using the LightCycler System

Cat. No. 12 015 137 001 Kit for 96 reactions

Store the kit at −15 to −25°C

⚠ Keep the LightCycler RT-PCR Reaction Mix SYBR Green I (vial 2) away from light!

Table of Contents

PROTOCOL

1. What this Product Does

Number of Tests The kit is designed for 96 reactions with a final reaction volume of 20 µl each.

Kit Contents

Vial/Cap	Label	Contents / Function
1 green cap	LightCycler RT-PCR Enzyme Mix	• 2× 20 µl • enzyme mix for RT-PCR
2 green cap	LightCycler RT-PCR Reaction Mix SYBR Green I, 5× conc.	• 3× 128 µl • reaction mix for RT-PCR • contains reaction buffer, dNTP mix (with dUTP instead of dTTP), SYBR Green I dye, and 15 mM $MgCl_2$
3 blue cap	$MgCl_2$ stock solution, 25 mM	• 1 ml • to adjust $MgCl_2$ concentration
4 colorless cap	H_2O, sterile-filtered, PCR grade	• 2× 1 ml • to adjust the final volume
5 colorless cap	Resolution Solution, 5×	• 1× 1 ml • to amplify RNA templates of high GC-content or high degree of secondary structures

Storage and Stability

The complete kit is stable through the expiration date printed on the label if stored properly at −15 to −25°C.

- The kit is shipped on dry ice.
- Once the kit is opened, store the kit components as described in the following table:

Vial	Label	Storage
1 green cap	LightCycler RT-PCR Enzyme Mix	• Store at −15 to −25°C. • **Avoid repeated freezing and thawing!**
2 green cap	LightCycler RT-PCR Reaction Mix SYBR Green I, 5× conc.	• Store at −15 to −25°C. • Aliquot the LightCycler RT-PCR Reaction Mix SYBR Green I • **Avoid repeated freezing and thawing!** • **Keep away from light!**

Vial	Label	Storage
3 blue cap	$MgCl_2$ stock solution, 25 mM	Store at −15 to −25°C.
4 colorless cap	H_2O, sterile-filtered, PCR grade	
5 colorless cap	Resolution Solution, 5×	

Additional Equipment and Reagents Required

Refer to the list below for additional reagents and equipment required to perform RT-PCR reactions with the LightCycler RNA Amplification Kit SYBR Green I using the LightCycler System:

- LightCycler System* (LightCycler 2.0 Instrument*, LightCycler 1.5 Instrument*, or an instrument version below)
- LightCycler Capillaries*
- Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes

  The LightCycler System provides adapters that allow LightCycler Capillaries to be centrifuged in a standard microcentifuge rotor.

or

- LightCycler Carousel Centrifuge 2.0* for use with the LightCycler 2.0 Carousel (optional)

  If you use a LightCycler Instrument version below 2.0, you need in addition the LightCycler Carousel Centrifuge 2.0 Bucket 2.1*. To adapt the LightCycler 2.0 Carousel to the former LightCycler Carousel Centrifuge, you need the LightCycler Carousel Centrifuge 2.0 Rotor Set*.
- LightCycler Control Kit RNA* (optional)
- Uracil-DNA N-Glycosylase, heat-labile*‡ (optional)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction (Eppendorf) tubes for preparing master mixes and dilutions

‡ for prevention of carry-over contamination; see section Related Procedures for details.

* available from Roche Applied Science; see Ordering Information for details.

Application

The LightCycler RNA Amplification Kit SYBR Green I is designed for use in research studies. The kit provides reagents, including RT-PCR enzyme mix, reaction mix, $MgCl_2$, Resolution Solution, and PCR-grade water for one-step RT-PCR in glass capillaries using the LightCycler System and SYBR Green I as detection format. Further, it can be used with heat-labile Uracil DNA N-Glycosylase to prevent carry-over contamination during PCR.

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 adapt each amplification protocol to the reaction conditions of the LightCycler System.

⚠ The amplicon size should not exceed 1 kb in length. For optimum results, select a product length of 700 bp or less.

⚠ The performance of the kit described in this Instruction Manual is guaranteed only when it is used with the LightCycler System.

2. How to Use this Product

2.1 Before You Begin

Sample Material

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. Higher concentrations might result in inhibition of the reaction.

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

For reproducible isolation of nucleic acids use:

- either the MagNA Pure LC Instrument together with a dedicated MagNA Pure LC reagent kit (for automated isolation)
- or a High Pure nucleic acid isolation kit (for manual isolation).

See Ordering Information for selected products recommended for isolation of template RNA. For further information consult the Roche Applied Science Biochemicals catalog or the website: www.roche-applied-science.com.

Primers

Use PCR primers at a final concentration of 0.3–1 µM. The recommended starting concentration is 0.5 µM each.

⚠ Refer to the LightCycler Operator's Manual for recommendations concerning the primer design.

$MgCl_2$

To ensure specific and efficient amplification with the LightCycler System, you must optimize the $MgCl_2$ concentration for each target. The LightCycler RT-PCR Reaction Mix SYBR Green I contains a $MgCl_2$ concentration of 3 mM (final concentration). The optimum concentration for RT-PCR with the LightCycler System may vary from 3 to 7 mM.

The table below shows the volume of the $MgCl_2$ stock solution (vial 3, blue cap) that you must add to a 20 µl reaction (final PCR volume) to increase the $MgCl_2$ concentration.

To reach a final Mg^{2+} concentration (mM) of:	3	4	5	6	7
Add this amount of 25 mM $MgCl_2$ stock solution (µl)	0	0.8	1.6	2.4	3.2

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template RNA with PCR-grade water (vial 4, colorless cap).

DNA Contamination Control

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

2.2 Procedure

LightCycler Protocol

The following procedure is optimized for use with the LightCycler System.

⚠ Program the LightCycler Instrument before preparing the reaction mixes.

A LightCycler protocol that uses the LightCycler RNA Amplification Kit SYBR Green I contains the following programs:

- **Reverse Transcription** of template RNA
- **Denaturation** of cDNA/RNA hybrid
- **Amplification** of the cDNA
- **Melting Curve** for amplicon analysis
- **Cooling** the rotor and thermal chamber

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For details on how to program the experimental protocol, see the LightCycler Operator´s Manual.

⚠ [1)] Temperature Transition Rate/Slope is 20°C/sec, except where indicated

⚠ Set all other protocol parameters not listed in the tables below to '0'.

The following table shows the PCR parameters that must be programmed for a LightCycler RT-PCR Run with the LightCycler RNA Amplification Kit SYBR Green I**.**

Analysis Mode	Cycles	Segment	Target Temperature [1)]	Hold Time	Acquisition Mode
Reverse Transcription					
None	1		55°C	10 min [4)]	none
Denaturation					
None	1		95°C	30 s	none
Amplification					
Quantification	45	Denaturation	95°C	0 s [5)]	none
		Annealing	primer dependent [2)]	10 s	none
		Extension	72°C [3)]	Product [bp] / 25 s [6)]	single
Melting Curve					
Melting Curves	1	Denaturation	95°C	0 s	none
		Annealing	65°C	10 s	none
		Melting	95°C slope = 0.1°C/sec [1)]	0 s	continuous
Cooling					
None	1		40°C	30 s	none

[2)] For initial experiments, set the target temperature (*i.e.,* the primer annealing temperature) 5°C below the calculated primer T_m.

[3)] If the primer annealing temperature is low (< 55°C), reduce the temperature transition rate/slope to 2–5°C/s.

[4)] When amplifying GC-rich templates or templates with a high degree of secondary structures, it is recommended to extend the incubation time to 30 min.

[5)] When amplifying GC-rich templates or templates with a high degree of secondary structures, it is recommended to extend the incubation time to 5 s.

[6)] 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.

Fluorescence and Run Setup Parameters

Parameter	Setting	
All LightCycler Software Versions		
Seek Temperature	55°C	
LightCycler Software prior to Version 3.5		
Display Mode	fluorescence channel F1	
Fluorescence Gains	**Fluorimeter**	**Gain Value**
	Channel 1 (F1)	3
	Channel 2 (F2)	1
	Channel 3 (F3)	1
LightCycler Software Version 3.5		
Display Mode	fluorescence channel F1	
Fluorescence Gains	not required In data created with LightCycler Software Version 3.5, all fluorescence values are normalized to a fluorescence gain of "1". This produces a different scale on the Y-axis than that obtained with previous LightCycler software versions. This difference does not affect the crossing points nor any calculated concentrations obtained.	
LightCycler Software Version 4.0		
Default Channel	fluorescence channel 530	
Fluorescence Gains	not required	
"Max. Seek Pos"	Enter the number of sample positions the instrument should look for.	
"Instrument Type"	• "6 Ch.": for LightCycler 2.0 Instrument (selected by default) • "3 Ch.": for LightCycler 1.5 Instrument and instrument versions below	
"Capillary Size"	Select "20 µl" as the capillary size for the experiment. ⚠ For the "6 Ch." instrument type only.	

Preparation of the 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.

❶ Depending on the total number of reactions, place the required number of LightCycler Capillaries in precooled centrifuge adapters or in a LightCycler Sample Carousel in a precooled LightCycler Centrifuge Bucket.

❷
- 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.

❸ Prepare a 10× conc. solution of PCR primers.

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

Component	Volume	Final conc.
H_2O, PCR-grade (vial 8, colorless cap)	x µl [2)]	–
LightCycler RT-PCR Reaction Mix SYBR Green I, 5× conc. (vial 2, green cap)	4.0 µl	1×
Resolution Solution, 5× conc. (vial 5, colorless cap)	y µl [2)]	0.5 – 1×
$MgCl_2$ stock solution (vial 3, blue cap)	z µl	Use concentration that is optimal for the target.
Primer Mix[1)], 10× conc.	2.0 µl	0.3 to 1.0 µM each (recommended conc. is 0.5 µM)
LightCycler RT-PCR Enzyme Mix (vial 1, green cap)	0.4 µl	
Total volume	**19 µl**	

To prepare the 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 + one additional reaction.

❺
- Mix gently by pipetting up and down. Do not vortex.
- Pipet 19 µl PCR mix into each LightCycler Capillary.
- Add 1 µl RNA template.
- Seal each capillary with a stopper.

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❺ • Place the 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 s (3000 rpm in a standard benchtop microcentrifuge).

• Alternatively, use the LightCycler Carousel Centrifuge for spinning the capillaries.

❼ Transfer the capillaries into the sample carousel of the LightCycler Instrument.

❽ Cycle the samples as described in section "LightCycler Protocol".

[1] Due to possible primer/primer interactions generated during storage it might be necessary to preheat the PCR primer mix for 1 min at 95°C before starting the reaction to achieve optimum sensitivity.

[2] When amplifying GC-rich templates or templates with high degree of secondary structures, use 2–4 µl Resolution Solution per 20 µl standard reaction. Adapt volume of water accordingly.

2.3 Related Procedures

Prevention of Carry-Over Contamination

Heat-labile Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination in PCR. This carry-over 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 in the table below to prevent carry-over contamination using heat-labile UNG:

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

❷ Add template RNA and incubate the completed reaction mixture for 5 min at room temperature.

❸ 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) since the reverse transcriptase would be inactivated.

When performing an additional melting curve analysis, the use of UNG lowers the respective melting temperature (T_m) by approx. 1°C.

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. 100 copies of the cytokine RNA can be reproducibly detected by amplifying in the LightCycler Instrument and using SYBR Green I as detection format.

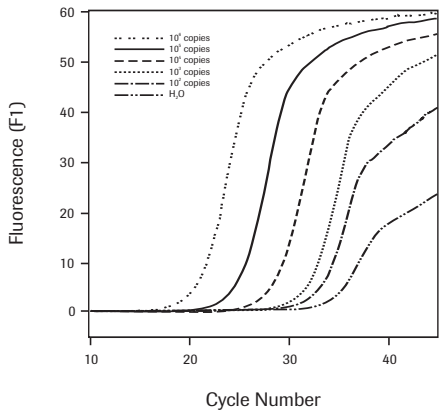

Fig. 1: Serially diluted samples containing 10^2 to 10^6 copies of cytokine RNA template from the LightCycler Control Kit RNA were amplified using the LightCycler RNA Amplification Kit SYBR Green I in a LightCycler Instrument. As a negative control, template RNA was replaced by PCR-grade water. Analysis was done using LightCycler Software 3.5. Arithmetic background subtraction was used and the fluorescence channel was set to F 1/1.

Melting Curve Analysis

Assess the specificity of the amplified PCR product by performing a melting curve analysis. The resulting melting curves allow 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.

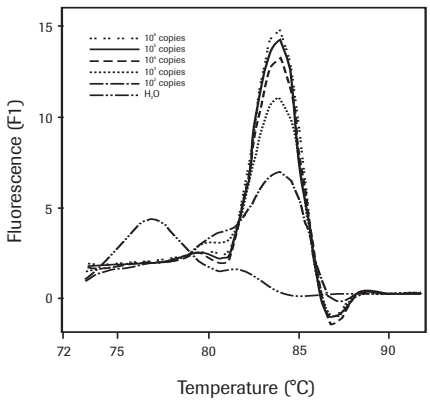

Fig. 2: Melting curve analysis of amplified samples containing 10^2–10^6 copies of cytokine RNA. As a negative control, the template RNA was replaced by PCR-grade water.

	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 room temperature. Mix gently from time to time until the precipitate is completely dissolved and place on ice.
Amplification reaches plateau phase before the program is finalized.	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 cycle program.
Log-linear phase of amplification just starts when the amplification program finishes.	Very low starting amounts of nucleic acid.	• Increase number of cycles by 10 in the corresponding cycle program. • Improve PCR conditions (*e.g.*, $MgCl_2$ concentration, primer and probe design). • Use higher amount of starting material. • Repeat the run.
	Number of programmed cycles is too low.	• Increase number of cycles in the corresponding cycle program.
No amplification occurs.	Wrong channel has been chosen to detect amplification online.	Check the channel chosen in the programming screen and change. (The data obtained up to this point will be saved.)
	Pipetting errors or omitted reagents.	• Check for missing reagents. • Titrate $MgCl_2$ concentration. • Check for missing or defective dye. • Check experimental protocol. • Always run a positive control along with your samples. • Check for missing or defective dye. • Increase amount of RNA template up to 1 mg total RNA or mRNA.
	Chosen gain settings are too low.	• Optimize gain setting using the **Real Time Fluorimeter** function.Gain settings cannot be changed during or after a run. Before repeating the run, check the gain settings in the cycle programs. ⚠ Gain settings do not have to be adjusted with LightCycler Software 3.5 or higher.
	Unsuitable scaling of the-graph.	• Change the values for the x- and y-axis by double-clicking on the maximum and/ or minimum values,and changing to suitable values.
	Measurements do not occur	Check the cycle programs. For SYBR Green I detection format, choose “single” as acquisition mode at the end of the elongation phase.
	Poor PCR efficiency due tounsuitable primers.	• Check PCR product on agarose gel. • Redesign primer. • Check annealing temperature of primers. • Primer concentration should be in the range of 0.3 to 1.0 μM.
	Amplicon length is >1 kb.	Do not use amplicons >1 kb. Amplification efficiency is reduced with amplicons of this size. Optimal results are obtained for amplicons up to 700 bp.
	Inhibitory effects of the sample material due to insufficient purification.	• Do not use more than 7-8 μl of RNA per 20 μl RT-PCR reaction mixture. • Repurify the nucleic acids to ensure removal of inhibitory agents.
	RNA degradation due to unproper storage or isolation.	• Check RNA quality on a gel. • Check RNA with an established primer pair if available.

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	Possible cause	Recommendation
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 Real Time Fluorimeter option to find suitable gain settings. The background fluorescence at measuring temperature should not exceed 10 for SYBR Green I. ⚠ Avoid bleaching of dyes by using an extra sample for this procedure. No gain setting required with LightCycler Software V3.5 and higher.
Fluorescence intensity is too low.	Deterioration of dyes in the reaction mixtures due to unsuitable storage conditions.	• Store the dye containing reagents at −15 to −25°C, protected from light. • Avoid repeated freezing and thawing.
	Chosen gain are too low.	Optimize gain setting using the Real Time Fluorimeter function. Change the gain settings in the cycle programs appropriately and repeat the run.
	Poor PCR efficiency due to non-optimized reaction conditions.	• Titrate $MgCl_2$ concentration • Primer concentration should be in the range of 0.3 to 1.0 μM, probe concentration should be in the range of 0.2 to 0.4 μM. • Check annealing temperature of primers and probes. • 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.
	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 min, and for denaturation during cycling to 5 s.
Fluorescence intensity varies.	Prepared PCR mix is still in the upper vessel of the capillary. Air bubble is trapped in the capillary tip.	Repeat centrifugation step.
	Skin oils on the surface of the capillary tip	Always wear gloves when handling the capillaries.
Amplification curve reaches plateau at a lower signal level than the other samples.	Too high starting amount of genomic RNA: RNA captures dye leading to a high background signal. Insufficient amounts of dye are left to monitor increase of fluorescence signal during amplification.	Use HybProbe detection format (max. amount of RNA: 1 μg).
	Dye bleached.	Check whether the reagents containing the dye have been stored protected from light. Avoid repeated freezing and thawing.
Negative control samples are positive.	Contamination	• Exchange all critical solutions. • Pipet reagents on a clean bench. • Close lid of the negative control reaction tube immediately after pipetting. • Use heat-labile UNG for decontamination of carry-over cross contamination.
Melting peak is very broad and peaks can not be differentiated.	°C to Average setting is too high.	• Lower the number of °C to Average. Only applicable for LightCycler Software versions prior to version 4.0.

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	Possible cause	Recommendation

Double melting peak appears for one product.	Two products of different length or GC content have been amplified (*i.e.*, pseudogenes or misprimming)	• 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.

Melting temperature of a product varies from experiment to experiment.	Variations in reaction mixture (e.g., salt concentration).	• Check purity of template solution. • Reduce variation of parameters such as $MgCl_2$, heat-labile UNG, and program parameters.
Only a primer dimer peak appears, and no specific PCR product peak, or very high primer dimer peaks.	Primer dimers have outcompeted amplification of specific PCR product.	• Keep all samples at 2-8°C (on ice) until the run is started. • The time between preparing the reac tion mixture and starting the run should be kept as short as possible. • See also recommendations under "Poor PCR efficiency".
	Insufficient quality of your primers.	Higher grade of primer purification.
	Unappropriate sequence of your primer.	Redesign primers.
Primer dimer and product peaks are very close together.	Unusual high GC-content of the primers.	• Redesign primers. • Run melting curve with lowest ramping rate (0.1 °C/s and continuous measurement). • Spread scale of the x-axis. • Lower the number of °C to Average.
Very broad primer dimer peak with multiple peaks.	Primer dimer variations (*e.g.*, concatameres, loops)	Redesign primers.
Only one identical peak of the same height in all samples.	Contamination in all samples.	• Close capillaries during centrifugation step. • Use fresh solutions.

5. Additional Information on this Product

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 System. It is used to perform one-step RT-PCR in 20 µl glass capillaries. Amplification and on-line monitoring of the template RNA is achieved by a combined procedure on the LightCycler Instruments. The results are interpreted directly after completing the PCR. The amplicon is detected by fluorescence using the double-stranded DNA-specific dye SYBR Green I.

The LightCycler RNA Amplification Kit SYBR Green I provides convenience, high performance, reproducibility, and minimizes contamination risk. Only template RNA, PCR primers, and $MgCl_2$ (if necessary), have to be added.

Background Information

Generation of PCR products can be detected by measurement of SYBR Green I fluorescence. SYBR Green I dye (already included in the Reaction Mix of the LightCycler RNA Amplification Kit SYBR Green I) will emit a fluorescence signal (wavelength, 530 nm) only when bound to double-stranded DNA. 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 non-specific products are present, they will be shown as additional melting peaks.

References

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Quality Control

The LightCycler RNA Amplification Kit SYBR Greeen I is function tested using the LightCycler Control Kit RNA.

6. Supplementary Information

6.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this Instruction Manual:

Text Convention	Usage
Numbered stages labeled ①, ②, etc.	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

6.2 Changes to Previous Version

- Information for usage of LightCycler Software 4.0 added.
- Standard protocol replaces protocol specific for the LightCycler Control Kit RNA.
- References describing product application added.

6.3 Ordering Information

Roche Applied Science 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, www.roche-applied-science.com and our Special Interest Sites including:

- The LightCycler System family for real-time, online PCR:
  http://www.lightcycler-online.com
- the MagNA Pure Family for automated nucleic acid isolation:
  http://www.magnapure.com
- manual nucleic acid isolation and purification:
  http://www.roche-applied-science.com/napure/

Instruments and Accessories

Product	Pack Size	Cat. No.
LightCycler 2.0 Instrument	1 instrument plus accessories	03 351 414 001
LightCycler 1.5 Instrument	1 instrument plus accessories	04 484 495 001
LightCycler Capillaries (20 µl)	1 pack (8 boxes, each with 96 capillaries and stoppers)	11 909 339 001
LC Carousel Centrifuge 2.0 Rotor Set	1 rotor + 2 buckets	03 724 697 001
LC Carousel Centrifuge 2.0 Bucket 2.1	1 bucket	03 724 689 001
LightCycler Carousel Centrifuge 2.0	1 centrifuge plus rotor and bucket	03 709 507 001 (115 V) 03 709 582 001 (230 V)
MagNA Pure LC Instrument	1 instrument plus accessories	12 236 931 001
LightCycler Software 4.0	1 software package	03 640 012 001
LightCycler Probe Design Software 2.0	1 software package	04 342 054 001

	Product	Pack Size	Cat. No.
RNA Isolation Kits	MagNA Pure LC RNA Isolation Kit – High Performance	1 kit (192 isolations)	03 542 394 001
	MagNA Pure LC RNA Isolation Kit III (Tissue)	1 kit (192 isolations)	03 330 591 001
	MagNA Pure LC mRNA Isolation Kit I (Blood, Blood Cells)	1 kit (192 isolations)	03 004 015 001
	MagNA Pure LC mRNA Isolation Kit II (Tissue)	1 kit (192 isolations)	03 172 627 001
	High Pure RNA Isolation Kit	1 kit (50 isolations)	11 828 665 001
	High Pure RNA Tissue Kit	1 kit (50 isolations)	12 033 674 001
	High Pure Viral RNA Kit	1 kit (100 isolations)	11 858 882 001
LightCycler One-Step RT-PCR Kits	LightCycler RNA Amplification Kit SYBR Green I	1 kit (96 reactions)	12 015 137 001
	LightCycler RNA Amplification Kit HybProbe	1 kit (96 reactions)	12 015 145 001
	LightCycler RNA Master SYBR Green I	1 kit (96 reactions)	03 064 760 001
	LightCycler RNA Master HybProbe	1 kit (96 reactions)	03 018 954 001
cDNA Synthesis Reagents	Transcriptor Reverse Transcriptase	250 U 500 U 2000 U	03 531 317 001 03 531 295 001 03 531 287 001
	Transcriptor First Strand cDNA Synthesis Kit	1 kit	04 379 012 001
	First Strand cDNA Synthesis Kit for RT-PCR (AMV)	1 kit	11 483 188 001
LightCycler Reagent Kits for Two-Step RT-PCR	LightCycler DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	12 015 102 001 12 158 825 001
	LightCycler FastStart DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	03 003 248 001 12 239 272 001
	LightCycler FastStart DNA Master^{PLUS} HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	03 515 575 001 03 515 567 001
	LightCycler DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	12 015 099 001 12 158 817 001
	LightCycler FastStart DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 003 230 001 12 239 264 001
	LightCycler FastStart DNA Master^{PLUS} SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 515 869 001 03 515 885 001
Associated Kits and Reagents	LightCycler Color Compensation Set	1 set (5 reactions)	12 158 850 001
	LightCycler Multicolor Demo Set	20 reactions & 5 color compensation runs	03 624 854 001
	LightCycler Control Kit RNA	1 kit (50 control reactions)	12 158 841 001
	Uracil-DNA N-Glycosylase, heat-labile	100 U	11 775 367 001
	RNA, MS2	10 A_{260} U (500 µl)	

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