For general laboratory use.

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LightCycler® Multiplex RNA Virus Master

Version 02

Content version: March 2014

Easy-to-use reaction mix for one-step RT-PCR using the LightCycler® 480, LightCycler® 96, or the LightCycler® Nano Real-Time PCR Systems

Cat. No. 06 754 155 001	Kit for 200 reactions (20 µl each)
Cat. No. 07 083 173 001	Kit for 1,000 reactions (20 µl each)

Store the kit at −15 to −25°C!

www.roche-applied-science.com

1. What this Product Does

Number of Tests

The kit is designed for

- 200 or 1,000 reactions with a final reaction volume of 20 µl each using the LightCycler® 480 System
- or the LightCycler® 96 System
- or the LightCycler® Nano System

Kit Contents

Vial/Cap	Label	Contents/Function A) Cat. No. 06 754 155 001 (200 reactions) B) Cat. No. 07 083 173 001 (1,000 reactions)
1 blue cap	RT-Enzyme Solution, 200× conc.	• A) 1 vial, 28 µl • B) 5 vials, 28 µl each, contains Reverse Transcriptase
2 red cap	RT-PCR Reaction Mix, 5× conc.	• A) 1 vial, 880 µl • B) 5 vials, 880 µl each, contains RT-PCR Reaction Buffer AptaTaq Polymerase, dATP, dCTP, dGTP, and dUTP, $MgCl_2$, and proprietary additives
3 colorless cap	Water, PCR grade	• A) 3 vials, 1 ml each • B) 15 vials, 1 ml each, to adjust the final reaction volume

Storage and Stability

Store the kit at −15 to −25°C.

For vial 1, close lid immediately after use.
For vial 2, avoid repeated freeze/thaw cycles (> 5×). To avoid repeated freeze/thaw cycles either aliquot vial 2 or store at +2 to +8°C.
The kit is stable at +2 to +8°C for 4 weeks.
The kit is shipped on dry ice.

Kit components are stable at -15 to -25°C until the expiration date printed on the label.

Although we recommend working on ice and preparing the reagents right before use, the working solution (everything combined except RNA template) is stable at +15 to +25°C up to 4 hours, and is therefore ideal for use in automated workflows.

Additional Equipment and Reagents Required

- Standard laboratory equipment
- Nuclease-free pipette tips
- 1.5 µl RNase-free microcentrifuge tubes to prepare master mixes and dilutions.
- To minimize risk of RNase contamination, autoclave all vessels.

Gloves should be worn at all times.

For RT-PCR:

- Real Time PCR systems such as LightCycler® 480 Instrument* LightCycler® 96 Instrument* and LightCycler® Nano Instrument*
- LightCycler® 480 Multiwell Plate 96* or 384*
- LightCycler® 8-Tube Strips*
- Standard swing-bucket centrifuge with rotor for multiwell plates

- For RT-PCR primer and probe design: Universal ProbeLibrary Assay Design Center at www.universalprobelibrary.com

Optional

For Virus RNA purification

- MagNA Pure 96 Instrument* including Disposables
- MagNA Pure 96 Internal Control Tube*, optional
- MagNA Pure 96 DNA and Viral NA Kit, Large Volume* or
- MagNA Pure 96 DNA and Viral NA Kit, Small Volume*

Alternatively use other nucleic acid purification instruments and manual sample preparation products such as:

- MagNA Pure LC 2.0 instrument* with MagNA Pure LC Total Nucleic Acid Isolation Kit - High Performance*
- MagNA Pure Compact instrument* with MagNA Pure Compact Nucleic Acid Isolation Kit I
- High Pure Viral Nucleic Acid Kit*
- FLOW System (for detailed information please refer to the Flow System User Training Guide)

Application

The LightCycler® Multiplex RNA Virus Master is designed for fast, highly sensitive and specific real-time one-step RT-PCR analysis of viral RNA.

The 2-vial composition (RT enzyme and mixture for PCR and RT-PCR) is ideally suited for use with RT minus controls. The proprietary reaction buffer allows a fast and convenient hot start RT-PCR without pre-activation of the Taq DNA Polymerase. The kit is optimized for hydrolysis probes, as well as Universal ProbeLibrary (UPL) probes and does not require optimization with $MgCl_2$.

Assay Time

The LightCycler® Multiplex RNA Virus Master can be used for multiplex RT-PCR protocols. For example, a triplex protocol using 45 cycles including a 10 min reverse transcription step requires 65 minutes, using the LightCycler® 480 System or 67 minutes using the LightCycler® 96 System.

2. How to Use this Product

2.1 Before You Begin

Precautions

Always use RNase-free techniques. RNase contaminated reagents and reaction vessels will degrade template RNA. Please follow these guidelines to minimize risk of contamination:

- Wear disposable gloves and change them frequently.
- Avoid touching surfaces or materials that could cause RNase carry-over.
- Use only reagents provided in this kit. Substitutions may introduce RNases.
- Clean and decontaminate work areas and instruments, including pipettes, with commercially available decontamination reagents.
- Use only new RNase-free aerosol-blocking pipette tips and microcentrifuge siliconized reaction tubes.
- Use a work area specifically designated for RNA work, and if possible use reaction vessels and pipettors dedicated only for work with template RNA.

Sample Material

Use any viral template RNA suitable for RT-PCR in terms of purity, concentration, and absence of RT-PCR inhibitors. For reproducible isolation of nucleic acids use:

- either the MagNA Pure LC Instrument*, the MagNA Pure Compact Instrument*, or the MagNA Pure 96 Instrument*, and a dedicated MagNA Pure Nucleic Acid Isolation Kit* (for automated isolation), or a High Pure Nucleic Acid Isolation Kit* (for manual isolation).
  For details see the Roche Applied Science catalogue or the website: www.roche-applied-science.com

Negative Control and RT Minus Control Reactions

Always run a negative control with the samples. To prepare a negative control, replace the template RNA with PCR grade water (Vial 3). A contamination problem can be observed using the negative control.

For the RT minus control, omit the RT enzyme in the mix. With the RT minus control, you can verify that your signal comes from the RNA target or from DNA contamination.

Primers

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

Probes

Suitable concentrations of hydrolysis probes range from 0.05 to 0.5 µM (final concentration in PCR). The recommended starting concentration is 0.25 µM each.

- The optimal probe concentration is the lowest concentration that results in the lowest quantification cycle value (Cq) and adequate fluorescence dynamics for a given target concentration.
- For a hydrolysis probe hybridization complex, the T_m of the hydrolysis probe has to be higher than the T_m of the primers.

$MgCl_2$

The master mix of this kit is optimized with a fixed concentration of $MgCl_2$, which works with nearly all primer combinations. There is no need for adjustment.

2.2 Procedure

LightCycler® 480, LightCycler® 96, LightCycler® Nano System Protocol

The following procedure is optimized for use with the corresponding LightCycler® System you are using.

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

A LightCycler® Instrument protocol that uses the LightCycler® Multiplex RNA Virus Master contains the following programs:

- **Reverse Transcription** of viral template RNA
- **Denaturation**: of cDNA/RNA hybrid
- **Amplification** of the cDNA
- **Cooling** of the thermal block

For details on how to program the experimental protocol, see the current LightCycler® 480 Instrument Operator's Manual, the LightCycler® 96 System Operator's Guide, or the LightCycler® Nano System Operator's Guide.

A) Protocol for use with the LightCycler® 480 Instrument II (Multiwell Plate 96 or 384)

The following table shows the parameters that must be programmed for a RT-PCR run using the LightCycler® Multiplex RNA Virus Master on the LightCycler® 480 Instrument II (Multiwell Plate 96 or 384).

Setup		
Detection Format	**Reaction Volume**	**Block Type**
For example: Monocolor Hydrolysis Probe /UPL Probe	20 µl	96 (384)
Programs		
Program Name	**Cycles**	Analysis Mode

Setup				
Reverse Transcription		1		None
Initial Denaturation		1		None
Cycling		45[1)]		Quantification
Cooling		1		None
Temperature Targets				
Target [°C]	**Acquisition Mode**	**Hold [hh:mm:ss]**	**Ramp Rate [°C/s]**	**Acquisitions [n/°C)]**
Reverse Transcription				
50[5)]	None	00:10:00[2)]	4.4 (4.8)	-
Initial Denaturation				
95	None	00:00:30	4.4 (4.8)	–
Amplification				
95	None	00:00:05	4.4 (4.8)	–
60[3)]	Single	00:00:30	2.2 (2.5)	–
Cooling				
40	None	00:00:30	2.2 (2.5)	-

[1)] 45 cycles are suitable for most assays. If the assay shows steep amplification curves and early crossing points, 40 cycles should be sufficient. Reducing the number of cycles will improve uniformity of the product and reduce the time required for the assay.

[2)] Ten minutes is the recommended default protocol. Depending on your assay, it should be possible to reduce the time for reverse transcription down to 4 minutes. For best results, run a test with 10, 8, 6, 4 minutes, etc.

[3)] Most available assays are designed for an annealing temperature of +60°C. If the Tm of the primers and probe indicate using different settings, select an annealing temperature +5°C below the calculated primer Tm for initial experiments. For assay optimization, choose the highest annealing temperature which still results in a high yield of amplicon with a low Cq and adequate fluorescence dynamics.

[5)] We recommend running the reverse transcription at +50°C. If necessary, it is also possible to run it at +55°C.

Color Compensation Protocol for the LightCycler® 480 Instrument II

For a multicolor measurement, the application of a color compensation file is necessary to compensate for optical crosstalk between the different channels. For the LightCycler® 480 Instrument II, an instrument-specific color compensation file is mandatory for multicolor experiments, and a color compensation object can be generated as shown below:

The LightCycler® 480 Instrument II protocol contains the following programs:

- **Reverse Transcription** of viral template RNA
- **Initial Denaturation** of cDNA/RNA hybrid
- **Amplification** of the cDNA
- **Temperature Gradient Step** to create the Color Compensation file
- **Cooling** of the thermal block

For details on how to program the experimental protocol, see the LightCycler® 480 Software Operator's Manual, version 1.5.

Programming a Customized Detection Format for the LightCycler® 480 System Filter Combination Selection

The Detection Format in the LightCycler® 480 Software, version 1.5 setup must be customized for the applied multicolor hydrolysis probe format used in the RT-PCR Detection. In the "Tool" module, the "Detection Formats" option allows creating a new detection format specified by the user, including a Detection Format list, a Filter Combination Selection area, and a Selected Filter Combination List. Different filter settings for the LightCycler® 480 II Instrument are defined.

Example for 3-color Hydrolysis Probes

Detection Formats	Excitation Filter	Emission Filter
FAM	465	510
Red 610	533	610
Cy5	618	660

For the new customized detection format, set for all selected filters in the "Selected Filter Combination List", the following values:

Melting Factor	1
Quantification Factor	10
Integration Time	2

The following table shows the RT-PCR parameters that must be programmed for a LightCycler® 480 Color Compensation file with a LightCycler® 480 Multiwell Plate 96.

Setup			
Detection Format	**Block Type**		
Customized (see section above)	96		
Programs			
Program Name	**Cycles**		Analysis Mode
Reverse Transcription	1		None
Initial Denaturation	1		None
Cycling	45		Quantification
Temperatur Gradient Step	1		Color Compensation
Cooling	1		None
Temperature Targets			
Target [°C]	**Acquisition Mode**	**Hold [hh:mm:ss]**	**Ramp Rate [°C/s]**
Reverse Transcription			
50	None	00:10:00	4.4
Initial Denaturation			
95	None	00:00:30	4.4
Amplification			
95	None	00:00:05	4.4
60	Single	00:00:30	4.4
Temperatur Gradient Step			
95	None	00:00:10	4.4
40	None	00:00:10	2.2
95	Continuous		5 Acq. /°C
Cooling			
40	None	00:00:30	2.2

Preparation of the Color Compensation Run

Prepare the calibrator RT-PCR mix for more than one reaction. Multiply the amount in the volume column by the number of reactions you need (minimum of 3 to 5 replicates) plus additional reactions since there will be a slight loss of liquid during the pipetting steps. In order to ensure accuracy, do not pipette volumes less than 1 µl.

For each dye, set up the following reactions:

Component	1× Buffer	1× for each Dye
RT-Enzyme Solution, 200× conc. (Vial 1)	0.1 µl	0.1 µl
RT-PCR Reaction Mix, 5× conc.(Vial 2)	4.0 µl	4.0 µl
Detection mix for each dye	-	X µl (depending on the assay)
Water, PCR grade (Vial 3)	15.9 µl	Y µl (depending on the assay)
Template, such as viral RNA or positive samples eluates	-	5 µl
Total Volume	**20.0 µl**	**20.0 µl**

❶	Pipette the three replicates of each different calibrator mix into a pre-cooled LightCycler® 480 Multiwell Plate 96.
❷	Seal the LightCycler® 480 Multiwell Plate with a LightCycler® 480 Sealing Foil.
❸	Place the Multiwell Plate in a standard swing-bucket centrifuge containing a rotor for multiwell plates with suitable adaptors. Balance it with a suitable counterweight (e.g., another plate), and centrifuge for 2 minutes at 1,500 × g.

Load the Multiwell Plate into the LightCycler® 480 Instrument and start the program.

Create Color Compensation Object

When the experiment is finished, open the **Sample Editor**. Select workflow **Color Comp** and designate the used positions of the Multiwell Plate as "Water" for Buffer replicates, and the appropriate dyes respectively (e.g., FAM, Red610, Cy5 for the example mentioned above).

Open the **Analysis** module Color Compensation, click on **Calculate**, and save the color compensation object in the database.

From this point on, you can apply this 'CC Object' to each multicolor experiment performed with FAM, Red610, and Cy5 on the same instrument.

B) Protocol for use with the LightCycler® 96 Instrument

Rund Editor			
Detection Format			**Reaction Volume**
For example: Dyes 1: FAM			20 µl
Programs			
Temp. [°C]	Ramp Rate [°C/s]	Hold [s]	Acquisition Mode
Preincubation (Reverse Transcription)			
50[5)]	4.4	600[2)]	None
Preincubation (Initial Denaturation)			
95	4.4	30	None
2-Step Amplification			
No. of Cycles: 45[1)]			
95	4.4	5	None
60[3)]	2.2	30	Single

[1)] 45 cycles are suitable for most assays. If the assay shows steep amplification curves and early crossing points, 40 cycles should be sufficient. Reducing the number of cycles will improve uniformity of the product and reduce the time required for the assay.

[2)] Ten minutes is the recommended default protocol. Depending on your assay, it should be possible to reduce the time for reverse transcription down to 4 minutes. For best results, run a test with 10, 8, 6, 4 minutes, etc.

[3)] Most available assays are designed for an annealing temperature of +60°C. If the Tm of the primers and probe indicate using different settings, select an annealing temperature +5°C below the calculated primer Tm for initial experiments. For assay optimization, choose the highest annealing temperature which still results in a high yield of amplicon with a low Cq and adequate fluorescence dynamics.

[5)] We recommend running the reverse transcription at +50°C. If necessary, it is also possible to run it at +55°C.

Color Compensation Protocol for the LightCycler® 96 Instrument

The LightCycler® 96 Instrument does not require the creation of a color compensation object,

C) Protocol for use with the LightCycler® Nano Instrument

Run Settings			
Optics Settings		**Reaction volume**	
For example: Hydrolysis Probes Normal Quality		20 µl	
Profile			
Programs			
Temp. (°C)	Ramp Rate[°C/s]	Hold [s]	Acquire
Hold (Reverse Transcription)			
50[5)]	5	600[2)]	-
Hold (Initial Denaturation)			
95	5	30	-
2-Step Amplification			
No. of Cycles: 45[1)]			
95	10[4)]	5	–
60[3)]	30	4	✓

[1)] 45 cycles are suitable for most assays. If the assay shows steep amplification curves and early crossing points, 40 cycles should be sufficient. Reducing the number of cycles will improve uniformity of the product and reduce the time required for the assay.

[2)] Ten minutes is the recommended default protocol. Depending on your assay, it should be possible to reduce the time for reverse transcription down to 4 minutes. For best results, run a test with 10, 8, 6, 4 minutes, etc.

[3)] Most available assays are designed for an annealing temperature of +60°C. If the Tm of the primers and probe indicate using different settings, select an annealing temperature +5°C below the calculated primer Tm for initial experiments. For assay optimization, choose the highest annealing temperature which still results in a high yield of amplicon with a low Cq and adequate fluorescence dynamics.

[4)] Note that for the LightCycler® Nano Instrument, it is not possible to program hold times shorter than 10 seconds. Program 10 seconds instead of 5 seconds for LightCycler® Nano Instruments.

[5)] We recommend running the reverse transcription at +50°C. If necessary, it is also possible to run it at +55°C.

Color Compensation Protocol for the LightCycler® Nano Instrument

The LC Nano Instrument does not require the creation of a color compensation object.

Setup of the PCR Reaction

Follow the procedure below to prepare at least ten 20 µl standard reactions:

Do not touch the surface of the LightCycler® 480 Multiwell Plate or the LightCycler® 8-Tube Strips.

1. Thaw the solutions and, to ensure recovery of all the contents, briefly spin vials in a microcentrifuge before opening.
   Mix carefully by pipetting up and down or vortex briefly. Place samples on ice.
2. Prepare a 20× conc. solution of your primers and a 20× conc. solution of your probes.
3. In a 1.5 ml reaction tube, prepare the RT-PCR Mix and put on ice.
   For best results, prepare not less than 10 reactions in order to reduce pipetting errors. To prepare more reactions, multiply the amount in the "Volume 1 Reaction" column below by the number of reactions to be run, plus at least one additional reaction.

Component	Volume 1 Reaction	Volume 10 Reactions	Final Conc.
Water, PCR grade (Vial 3)	8.9 µl	89 µl	-
RT-PCR Reaction Mix, 5× (Vial 2)	4 µl	40 µl	1×
Primer Mix, 20×[1)]	1 µl	10 µl	1×
Probe Mix, 20×	1 µl	10 µl	1×
RT-Enzyme Solution, 200×	0.1 µl	1 µl	1×
Total volume	**15 µl**	**150 µl**	

   [1)] Due to possible primer/primer interactions that occur during storage, it may be necessary to preheat the PCR primer mix for 1 minute at +95°C before pipetting the complete mixture. This extra step will ensure optimum sensitivity.

4. Mix carefully by pipetting up and down or vortex briefly. Place on ice.
   Although we recommend working on ice and preparing the reagents right before use, the working solution (everything combined except RNA template) is stable at +15 to +25°C up to 4 hours, and is therefore ideal for use in automated workflows.
5. Prepare sample concentration of the viral RNA and/or DNA.
6. Pipette 15 µl RT-PCR Mix into a precooled multiwell plate or precooled LightCycler® 8-Tube Strip.
   Add 5 µl of the RNA and/or DNA template.
   Seal multiwell plate with LightCycler® 480 Sealing Foil or LightCycler® 8-Tube Strips using the corresponding lid.
7. Place the Multiwell Plate 96 into a standard swing-bucket centrifuge with a suitable adapter and balance it with a suitable counterweight (*e.g.,* another multiwell plate), or place the 8-Tube Strips into a standard Multiwell Plate 96 and balance them in the centrifuge.
   Centrifuge at 1,500 × g for 0.5 - 2 minutes.

8. Load the reaction vessels into the LightCycler® 480, LightCycler® 96, or LightCycler® Nano Instrument.
9. Start the PCR program described above.
   If you use reaction volumes other than 20 µl, be sure to adapt the right volume in the running protocol. To start, we recommend using the same hold times as for the 20 µl volume.

2.3 Quality Control

Each lot of LightCycler® Multiplex RNA Virus Master is tested to meet specifications of the RT-PCR using a duplex RT-PCR assay on the LightCycler® 480 Instrument.

3. Results

The following results were obtained using the LightCycler® Multiplex RNA Virus Master on the LightCycler® 480 Instrument. A duplex reaction using primers and UPL probes specific for G6PD (FAM) and ß2M (Yellow555) was run. Human total RNA served as the template. Total RNA from different tissues in a dilution series [50 ng, 5 ng, 500 pg, 50 pg, and 10 pg and a no template control (NTC)] was analyzed using real-time PCR.

FAM channel (465/510)

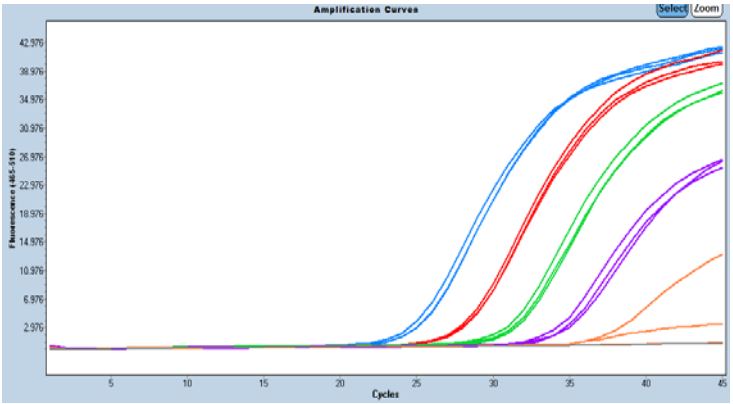

Fig. 1: The FAM channel shows the results for G6PD. Amplification curves shown were obtained from dilutions of 50 ng (blue), 5 ng (red), 500 pg (green), 50 pg (purple), and 10 pg (orange) human total RNA per well, including a no template control (grey). Duplex RT-PCR was performed in a reaction volume of 20 µl per well in a LightCycler® 480 Multiwell Plate 96.

Yellow555 channel (533/580)

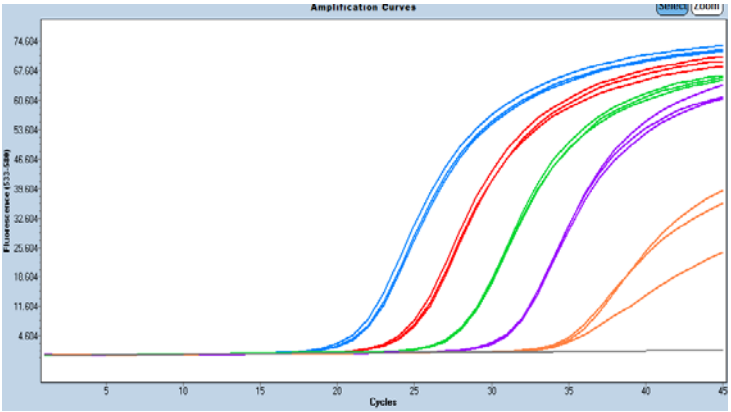

Fig. 2: The Yellow555 channel shows the results for ß2M. Amplification curves shown were obtained from dilutions of 50 ng (blue), 5 ng (red), 500 pg (green), 50 pg (purple), and 10 pg (orange) human total RNA per well, including a no template control (grey). Duplex RT-PCR was performed in a reaction volume of 20 µl per well in a LightCycler® 480 Multiwell Plate 96.

4. Troubleshooting

	Possible Cause	Recommendation
Increase Specificity		Some assays show higher specificity when using a higher reverse transcription temperature and/or higher annealing temperature.
Fluorescence intensity varies	Some of the reagent is still in the upper part of the microwell/8-tube strip, or an air bubble is trapped in microwell/8-tube strip.	Repeat centrifugation, but allow sufficient centrifugation time so all reagent is at the bottom of the microwell/8-tube strip and air bubbles are expelled.
	Skin oils or dirt on the surface of the microwell/ 8-tube strip.	Always wear gloves when handling the multiwell plate/8-tube strip.
Fluorescence intensity is very low	Low concentration or deterioration of dyes in the reaction mixtures because dye was not stored properly.	• Keep dye-labeled reagents away from light. • Store the reagents at −15 to −25°C and avoid repeated freezing and thawing.
	Poor PCR efficiency (reaction conditions not optimized)	• Primer concentration should be in the range of 0.2 to 0.5 μM, probe concentration should be in the range of 0.2 to 0.5 μM and half of the primer concentration. • Check annealing temperature of primers and probes. • Check experimental protocol. • Optimize annealing temperature in the reverse transcription step or in the PCR reaction. • Always run a positive control along with your samples.
	Chosen imaging time is too low	• Choose the appropriate detection format in combination with “dynamic” detection mode or • Increase imaging time when using “manual” detection mode. For details see LightCycler® 480 Software Instrument Operator's Manual.
	RT-PCR primers and probes are not optimized	• Check sequence and location of the hydrolysis probe on the PCR product. • Check RT-PCR product on an agarose gel
	PCR has not been optimized	• Check primer design (quality). • Check RT-PCR product on an agarose gel

	RNA is degraded during isolation or improper storage	• If possible check RNA quality. • Check RNA with an established RT-PCR primer when available
	Pipetting errors and omitted reagents	• Check for missing reagents • Check the pipetting procedure
	Impure sample material inhibits reaction	• Dilute sample 1:10 and repeat the analysis. • Repurify the nucleic acids to ensure removal of inhibitory agents.
Negative control sample gives a positive signal	Contamination	Remake all critical reaction mixes. Please use special RT-PCR setup working areas.

5. Additional Information on this Product

How this Product Works

The LightCycler® Multiplex RNA Virus Master consists of 3 different vials:

- Vial 1: Reverse Transcriptase
- Vial 2: RT-PCR Reaction Mix
- Vial 3: Water, PCR grade

The separate vial of Reverse Transcriptase makes it possible to prepare a RT-PCR reaction mix for running RT minus controls (lacking reverse transcriptase). This is important for verifying that the obtained results are derived from RNA transcripts or from residual (contaminating) genomic DNA. The kit also provides sufficient numbers of vials containing PCR grade water to ensure that fresh (unopened) vials can be used. This minimizes the risk of contamination of RT-PCR reaction mixes with RNases and other substances.

The Reverse Transcriptase provided in this kit is a recombinant reverse transcriptase with higher stability than native reverse transcriptase. This feature allows for higher reverse transcription temperatures up to +55°C. This Roche recombinant Reverse Transcriptase also has lower affinity for DNA than other commonly used reverse transcriptases.

The resulting RT-PCR reaction mix also has Taq DNA Polymerase, nucleotides, and additives ensuring a hot start amplification system with high specificity. The mix contains an optimized concentration of $MgCl_2$, eliminating the need for additional adjustments. For greater convenience, the RT-PCR mixture, including Reverse Transcriptase, can be used for both RNA and/or DNA templates in parallel.

6. Supplementary Information

6.1 Conventions

6.1.1 Text Conventions

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

Text Convention	Usage
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.

6.1.2 Symbols

In this document, 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

- Editorial changes.

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 home page, www.roche-applied-science.com, and our Special Interest Sites for:

- the LightCycler® 480 System: www.lightcycler480.com
- LightCycler® 96 System: www.lightcycler96.com
- Automated Sample Preparation (MagNA Pure LC System and MagNA Pure Compact System): www.magnapure.com
- Manual Sample Preparation of Nucleic Acids: www.roche-applied-science.com/shop/en/us/products/manual-sample-preparation-of-nucleic-acids

Instrument and Accessories

Product	Pack Size	Cat. No.
LightCycler® 480 Instrument II, 96 well	1 instrument (96 well)	05 015 278 001
LightCycler® 480 Instrument II, 384 well	1 instrument (384 well)	05 015 243 001
LightCycler® 480 Block Kit 96 Silver	1 block kit for 96-well PCR Multiwell Plates	05 015 219 001
LightCycler® 480 Block Kit 384 Silver	1 block kit for 384 -well PCR Multiwell Plates	05 015 197 001
LightCycler® 480 Multiwell Plate 96, white (Use with the LightCycler® 480 Instrument II, 96 well, and the LightCycler® 96 Instrument)	5 × 10 plates with sealing foils	04 729 692 001
LightCycler® 480 Multiwell Plate 384, white (Use with the LightCycler® 480 Instrument II, 384 well)	5 × 10 plates with sealing foils	04 729 749 001
LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
LightCycler® 480 Software, Version 1.5	1 software package	04 994 884 001
LightCycler® 96 Instrument	1 instrument	05 815 916 001

	Product	Pack Size	Cat. No.
	LightCycler® Nano Instrument	1 instrument	06 407 773 001
	LightCycler® 8-Tube Strips (white)	10 x 12 white strips and clear caps. Each pack of LightCycler® 8-Tube Strips contains 10 × 12 strips of both tubes and flat caps, in 10 non-sterile plastic bags	06 612 601 001
	LightCycler® 8-Tube Strips (clear) (Use with the LightCycler® Nano Instrument)	10 x 12 clear strips and clear caps. Each pack of LightCycler® 8-Tube Strips contains 10 × 12 strips of both tubes and flat caps, in 10 non-sterile plastic bags	06 327 672 001
	LightCycler® 8-Tube Strip Adapter Plate		06 612 598 001
	MagNA Pure 96 Instrument	1 instrument plus accessories	06 541 089 001
	MagNA Pure 96 Internal Control Tube	150 tubes (15×10)	05 435 293 001
	MagNA Pure LC 2.0 Instrument	1 instrument plus accessories	05 197 686 001
	MagNA Pure Compact Instrument	1 instrument plus accessories	03 731 146 001
RNA Virus Isolation Kits	MagNA Pure 96 DNA and Viral NA Small Volume Kit	3 sets for 192 isolations each	06 543 588 001
	MagNA Pure 96 DNA and Viral NA Large Volume Kit	3 sets for 96 isolations each	06 374 891 001
	MagNA Pure 96 Cellular RNA Large Volume Kit	3 sets for 96 isolations each	05 467 535 001
	MagNA Pure LC Total Nucleic Acid Isolation Kit - High Performance	1 kit for up to 288 isolations	05 323 738 001
	MagNA Pure LC Total Nucleic Acid Isolation Kit	1 kit (192 isolations)	03 038 505 001
	MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume	1 kit (192 isolations)	03 264 793 001
	MagNA Pure LC RNA Isolation Kit - High Performance	1 kit (192 isolations)	03 542 394 001
	MagNA Pure Compact Nucleic Acid Isolation Kit I	1 kit for 32 isolations	03 730 964 001
	MagNA Pure Compact RNA Isolation Kit	1 kit for 32 isolations	04 802 993 001

Product	Pack Size	Cat. No.
High Pure Viral RNA Kit	1 kit for up to 100 purifications	11 858 882 001
High Pure Viral Nucleic Acid Kit	1 kit for up to 100 purifications	11 858 874 001

6.4 Disclaimer of License

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