For general laboratory use.

RealTime ready RNA Virus Master

Version: 10

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Easy-to-use reaction mix for one-step RT-PCR using with the LightCycler® 480 System or the LightCycler® Carousel-Based System

Cat. No. 05 619 416 001	1 kit 100 reactions of 20 µl final volume each
Cat. No. 05 992 877 001	1 kit 1,000 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	Catalog Number	Content
1	red	Enzyme Blend, 50x conc.	Contains Transcriptor RT, Taq DNA Polymerase and proprietary additives. No activation at +95°C is necessary.	05 619 416 001	1 vial, 40 µl
				05 992 877 001	10 vials, 40 µl each
2	colorless	Reaction Buffer, 5x conc.	Contains RT-PCR Reaction Buffer, dATP, dCTP, dGTP and dUTP, $MgCl_2$, and proprietary additives. *Keep vial 2 away from light!*	05 619 416 001	1 vial, 400 µl
				05 992 877 001	10 vials, 400 µl each
3	colorless	Water, PCR grade	To adjust the final reaction volume.	05 619 416 001	1 vial, 1 ml
				05 992 877 001	10 vials,1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped on dry ice.
The kit components are stable at -15 to -25°C until the expiration date printed on the label.

Store the kit components as follows:

Vial / Bottle	Cap	Label	Storage
1	red	Enzyme Blend, 50x conc.	Store in the provided plastic can at -15 to -25°C.
2	colorless	Reaction Buffer, 5x conc.	

1.3. Additional Equipment and Reagent required

- Standard laboratory equipment
- Nuclease free pipette tips
- 1.5 ml RNase-free microcentrifuge tubes for preparing master mixes and dilutions
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To minimize risk of RNase contamination, autoclave all vessels

⚠ ***Gloves should be worn at all times.***

For qPCR:
- Real Time PCR systems such as LightCycler® 480 Instrument (96-well)*
- LightCycler® 2.0 Instrument* and LightCycler® 1.x Instrument*
- LightCycler® 480 Multiwell Plate 96*
- LightCycler® Capillaries (20 µl)*
- Standard swinging-bucket centrifuge with rotor for multiwell plates

For qPCR primer and probe design:

- Universal ProbeLibrary Assay Design Center at: www.universalprobelibrary.com

Optional
For Virus RNA purification
- MagNA Pure LC Total Nucleic Acid Isolation Kit - High Performance*
- MagNA Pure LC Total Nucleic Acid Isolation Kit*
- MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume*
- MagNA Pure LC RNA Isolation Kit - High Performance*
- MagNA Pure Compact Nucleic Acid Isolation Kit I*
- MagNA Pure Compact Nucleic Acid Isolation Kit I - Large Volume*
- MagNA Pure Compact RNA Isolation Kit*
- High Pure Viral RNA Kit*
- High Pure Viral Nucleic Acid Kit*
- High Pure Viral Nucleic Acid Large Volume Kit*

1.4. Application

The RealTime ready RNA Virus Master is designed for fast, high sensitive and specific real-time one-step RT-PCR analysis of viral RNA.

With its 50-fold concentrated mixture of Transcriptor and Taq DNA Polymerase, a 5-fold reaction buffer containing dNTPs and $MgCl_2$, the formulation is suited for the detection of viral RNA.

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 Hybridization probes, Hydrolysis probes, as well as for Universal ProbeLibrary (UPL) probes, and does not require optimization with $MgCl_2$.

1.5. Preparation Time

Assay Time

Typical Run Time

The RealTime ready RNA Virus Master can be used for fast RT-PCR protocols with run times of 25 - 50 min using a LightCycler® 480 System or a LightCycler® Carousel-Based System.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any viral template RNA suitable for RT-qPCR in terms of purity, concentration, and absence of RT-PCR inhibitors.

For reproducible isolation of nucleic acids, use:
- 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

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with Water, PCR-grade (vial 3). A contamination problem can be observed using the negative control.

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.

Probe

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

The optimal probe concentration is the lowest concentration that results in the lowest crossing point (Cp) value and adequate fluorescence dynamics for a given target concentration.

For a digestible hybridization complex, the Tm of the hydrolysis probe has to be higher than the Tm of the primers.

Mg2+ Concentration

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.

General Considerations

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 carryover.
- 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 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.

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

Protocol for Use with the LightCycler® 480 System

The following procedure is optimized for use with the LightCycler® 480 System.

⚠ ***Program the LightCycler® 480 Instrument before preparing the reaction mixes.***
A protocol for use with LightCycler® 480 Instrument that uses RealTime ready RNA Virus Master contains the following programs:

- **Reverse Transcription** of viral template RNA
- **Pre-Incubation** for activation of DNA polymerase and 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 LightCycler®480Software, Version 1.5 Manual.

Protocol for Use with the LightCycler® 480 Multiwell Plate 96

The following table shows the RT-PCR parameters that must be programmed for a LightCycler® 480 System RT-PCR run with the RealTime ready RNA Virus Master using a LightCycler®480 Multiwell Plate 96.

Setup		
Block Type	Reaction Volume [µl]	
96	20	
Detection Format	Excitation Filter	Emission Filter
Monocolor Hydrolysis Probe / UPL Probe FAM	465	510
or Monocolor HybProbe Red 640	498	640
For new customized detection formats, set for all selected filters in the “Selected Filter Combination List” (under Tools), the following values:		
Melt Factor	1	
Quant Factor	10	
Max Integration Time [s]	2	
Programs		
Program Name	Cycles	Analysis Mode
Reverse Transcription	1	None
Pre-Incubation	1	None
Amplification	45 [1)]	Quantification
Cooling	1	None

Temperature Targets					
	Target [°C]	Acquisition Mode	Hold [hh:mm:ss]	Ramp Rate [°C/s]	Acquisitions [per °C]
Reverse Transcription	50[2)]	None	00:08:00	4.4	-
Pre-Incubation	95	None	00:00:30	4.4	-
Amplification	95	None	00:00:01	4.4	-
	60[3)]	Single	00:00:20	2.2	-
	72	None	00:00:01	4.4	-
Cooling	40	None	00:00:30	1.0	-

(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) Depending on the Tm of the reversed primer.
(3) Most of the available assays are designed for an annealing temperature of +60°C. If the Tm of the primers and probe afford 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 Cp and adequate fluorescence dynamics.

Protocol for Use with the LightCycler® Carousel-Based System

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

⚠ ***Program the LightCycler® 2.0 or 1.x instrument before preparing the reaction mixes.***
A protocol for use with LightCycler® 2.0 or 1.x Instrument that uses RealTime ready RNA Virus Master contains the following programs:
- **Reverse Transcription** of viral template RNA
- **Pre-Incubation** for activation of DNA polymerase and denaturation of cDNA/RNA hybrid
- **Amplification** of the cDNA
- **Cooling** the rotor and thermal chamber

For details on how to program the experimental protocol, see the LightCycler® Software 4.1 Manual.

The following table shows the PCR parameters that must be programmed for a LightCycler® Carousel-Based PCR run with the RealTime ready RNA Virus Master using LightCycler® Capillaries (20 µl).

LightCycler® Software Version 4.1	
Programs	
Setup	Setting
Default Channel	Fluorescence Channel
Seek Temperature	30°C
Max Seek Pos.	Enter the total number of sample positions for which the instrument should look for.
Instrument Type	“6 Ch.” for LightCycler® 2.0 Instrument or “3 Ch.” for LightCycler® 1.5 Instrument and lower instrument versions
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[1)]	Quantification	
Cooling		1	None	
Temperature Targets				
	Target [°C]	Hold [hh:mm:ss]	Ramp Rate [°C/s]	Acquisition Mode [per °C]
Reverse Transcription	50 [2)]	00:08:00	20	None
Pre-Incubation	95	00:00:30	20	None
Amplification	95	00:00:01	20	None
	60 [3)]	00:00:20	20	Single
	72	00:00:01	2	None
Cooling	40	00:00:30	20	None

(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) Depending on the Tm of the reversed primer.
(3) Most of the available assays are designed for an annealing temperature of +60°C. If the Tmof the primers and probe afford 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 Cp and adequate fluorescence dynamics.

Setup of the RT-PCR Reaction

Follow the procedure below to prepare one 20 µl standard reaction:

⚠ ***Do not touch the surface of the LightCycler® 480 Multiwell Plate or the LightCycler® Capillaries.***

1. Thaw vials 1 to 3 of the Enzyme Blend, Reaction Buffer and Water, PCR grade. To ensure recovery of all the contents, briefly spin vials 1 and 2 in a microcentrifuge before opening and mix carefully by pipetting up and down.

2. Prepare a 20x concentrated specific PCR primers and probes mixture.

3. Prepare a sample concentration of the viral RNA samples of 10 to 10^6 copies/x µl.

4. Dispense equal amounts of reaction mixture and sample dilutions to the respective wells of the LightCycler® 480 Multiwell Plate 96 or LightCycler® Capillaries (*e.g.*, 5 µl RNA virus template each for a reaction volume of 20 µl).

5. Seal the LightCycler® 480 Multiwell Plate 96 plate with adequate sealing film, or when using the LightCycler® 1.x or 2.0 Instrument seal each LightCycler® Capillary with a stopper.

6. Place the multiwell plate in a standard swinging-bucket centrifuge, containing a rotor for multiwell plates with suitable adaptors, balance it with a suitable counterweight (*e.g.*, another multiwell plate), and centrifuge for 2 min at 1500×*g*.
   When using the LightCycler®1.xor2.0Instrument: If a LCCarouseCentrifuge is available, spin the capillaries in a LightCycler®SampleCarousel in the LightCycler®CarouselCentrifuge.
   Alternatively, place the capillaries in cooled adapters in a standard benchtop microcentrifuge, centrifuge at 700×g (3,000rpm) for 5s, and transfer the capillaries to the LightCycler®SampleCarousel.
   ℹ *Place the centrifuge adapters in a balanced arrangement within the centrifuge.*

7 Load the multiwell plate into the LightCycler® 480 Instrument. Or alternatively, place the LightCycler® Sample Carousel in the LightCycler®Carousel-Based System.

8 Start the PCR program.

In a 1.5 ml reaction tube on ice, prepare the RT-PCR Mix per 20 µl reaction by adding the following components in the order mentioned below, then mix gently up and down:

Component Volume for a 20 µl reaction

Pipetting Protocol	µl per 20 µl reaction	Final concentration
Water, PCR grade	to a total final reaction volume of 20 µl	
Enzyme blend, 50x conc.	0.4 µl	1x
Reaction buffer, 5x conc.	4 µl	1x
Primer 1, 10 µM	1 µl	0.5 µM
Primer 2, 10 µM	1 µl	0.5 µM
Probe, 10 µM	1 µl	0.5 µM
Sample / viral RNA	x µl	approx. 10 to 10^6 copies

⚠ ***To prepare the RT-PCR mix for more than one reaction, multiply the amount in the "Volume" column above by the number of reactions. Please note that there will be a slight loss of liquid during the pipetting steps. Please calculate extra volume of RT-PCR mix by adding one reaction volume.***

For a 20 µl reaction:
Pipette 15 µl RT-PCR mix into each precooled LightCycler® Capillary or LightCycler® 480 Multiwell Plate 96 and add 5 µl of the RNA virus template.

3. Results

The following results were obtained using the RealTime ready RNA Virus Master on the LightCycler® 480 Instrument using primers and hybridization probe specific for Hepatitis A Virus (HAV), and dilutions of viral template RNA.

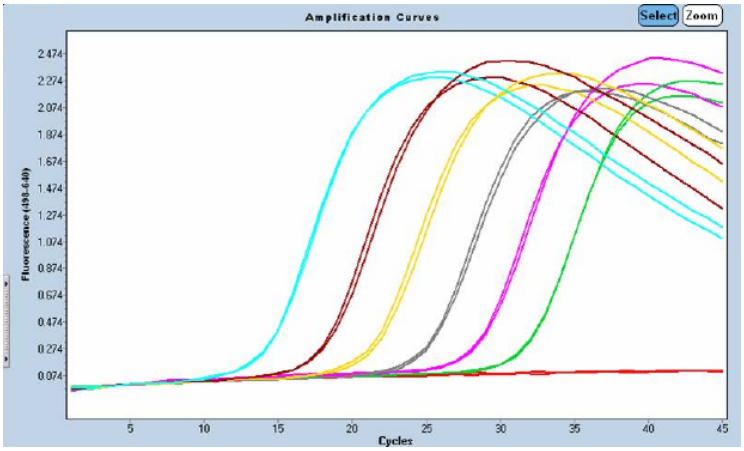

Fig. 1: Amplification curves obtained from dilutions of 10^6 [far left] to 10 [far right] target HAV viral RNA copies per well. RT-PCR was performed in a reaction volume of 20 µl per well in a LightCycler® 480 Multiwell Plate 96. The results confirm the high sensitivity and linearity of the RealTime ready RNA Virus Master.

4. Troubleshooting

Observation	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/capillary, or an air bubble is trapped in microwell/ capillary.	Repeat centrifugation, but allow sufficient centrifugation time so all reagent is at the bottom of the microwell/capillary and air bubbles are expelled.
	Skin oils or dirt on the surface of the microwell/capillary.	Always wear gloves when handling themultiwell plate/capillary.
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.
		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 Roche Detection Format in combination with “dynamic” detection mode,or ▪ Increase imaging time when using “manual” detection mode. For details see LightCycler®480 Software, Version 1.5 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.	Check RNA quality on a gel.
		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.	Change the values for the x- and the y-axis by double-clicking on the maximum and/or minimum values, then changing to suitable values.
		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

5.1. Quality Control

Each lot of RealTime ready RNA Virus Master is tested to meet specifications of qRT-PCR using *in vitro* transcribed Hepatitis A Virus RNA (HAV).

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

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.

Product	Pack Size	Cat. No.
Accessories general (hardware)		
LC Carousel Centrifuge 2.0		12 189 682 001
	1 centrifuge plus rotor and rotor bucket (115 V)	03 709 507 001
	1 centrifuge plus rotor and rotor bucket (230 V), *Not available in US*	03 709 582 001
LightCycler® Software 4.1	1 software package	04 898 915 001
LightCycler® 480 Block Kit 96 Silver	1 block kit	05 015 219 001
Accessories software		
LightCycler® 480 Software, Version 1.5	1 software package	04 994 884 001
Consumables		
LightCycler® 480 Multiwell Plate 96, white	5 x 10 plates	04 729 692 001
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
LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
Instruments		
LightCycler® 480 Instrument II	1 instrument	05 015 278 001
	1 instrument	05 015 243 001
Reagents, kits		
MagNA Pure LC Total Nucleic Acid Isolation Kit - High Performance	1 kit, up to 288 isolations	05 323 738 001
High Pure Viral RNA Kit	1 kit, up to 100 purifications	11 858 882 001
MagNA Pure Compact Nucleic Acid Isolation Kit I - Large Volume	1 kit, 4 x 8 sealed cartridges, 32 isolations	03 730 972 001
MagNA Pure Compact RNA Isolation Kit	1 kit, 4 x 8 sealed cartridges, 32 isolations	04 802 993 001
MagNA Pure Compact Nucleic Acid Isolation Kit I	1 kit, 4 x 8 sealed cartridges, 32 isolations	03 730 964 001
High Pure Viral Nucleic Acid Large Volume Kit	1 kit, 40 isolations	05 114 403 001
MagNA Pure LC RNA Isolation Kit - High Performance	1 kit, up to 192 isolations	03 542 394 001
MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume	1 kit, up to 192 isolations	03 264 793 001

6.4. Trademarks

HYBPROBE, HIGH PURE, MAGNA PURE and LIGHTCYCLER are trademarks of Roche.
All other 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 general laboratory use.

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
- Information Material

To call, write, fax, or email us, visit **lifescience.roche.com** and select your home country to display country-specific contact information.

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany