LightMix® Kit *RVF Virus*

Cat.-No. 40-0360-16

Kit with reagents for the detection of *RVF Virus* cDNA using the LightCycler® 1.x / 2.0 Instruments.

Lyophilized mix of primers and probes (6 tubes with 16 rxns each) for a total of 96 reactions with a final volume of 20 µl each. **Store protected from light at room temperature (18-25°C), do NOT freeze!**

Additional reagents required

Roche Diagnostics:	
LightCycler® FastStart DNA Master HybProbe	Cat.-No. 03 003 248 001
LightCycler® Color Compensation Set (LightCycler® 1.x Instrument)	Cat.-No. 12 158 850 001
or LightCycler® Multicolor Demo Set	Cat.-No. 03 624 854 001
High Pure Viral Nucleic Acid Kit	Cat.-No. 11 858 874 001
Transcriptor First Strand cDNA Synthesis Kit	Cat.-No. 04 379 012 001

1. Introduction

Rift Valley Fever Virus (RVF Virus) is a lipid-enveloped RNA virus which belongs to the family Bunyaviridae genus Phlebovirus. The viral genome is tripartite-segmented and contains single-stranded negative-sense and ambisense RNA's (L, M and S). Rift valley fever virus is transmitted by mosquitoes or contact with infected materials and affects domestic animals (cattle, buffalo, sheep, goats and camels) as well as humans. In humans, it usually causes an influenza-like disease but occasionally leads to more serious complications with high morbidity and mortality like ocular diseases, meningoencephalitis or haemorrhagic fever.

The LightMix® Kit *RVF Virus* provides a fast, easy and accurate system to identify this target in a nucleic acid extract. A control amplification reaction acts as internal positive control (IPC).

This LightMix® Kit is tested with the Roche Diagnostics 'LightCycler® FastStart DNA Master HybProbe' in the LightCycler® 1.x / 2.0 Instruments. A 1-step RT PCR procedure using the Roche 'LightCycler® 480 RNA master' was tested (start with 3 min 63°C R T step before denaturation, 95°C step to 1 min).

2. Description

A 189 bp fragment of the *RVF Virus* cDNA is amplified with specific primers. The resulting PCR fragment is analyzed with hybridization probes labeled with LightCycler® Red 640 (detected in channel 640). The PCR product is identified by running a melting curve with a specific melting point (Tm). The *RVF Virus* cDNA exhibits a Tm of 68°C in channel 640.

The PCR reaction is monitored by an additional PCR product of 278 bp, formed from the internal positive control. This control will not interfere with the *RVF Virus* specific reactions. The amplification will usually fail in the presence of higher concentrated *RVF Virus* cDNA samples (1,000 - 10,000 copies or higher) while displaying an amplification signal in negative and low-concentrated samples. The hybridization probes are labeled with LightCycler® Red 705. Detection is recorded in channel 705. The IPC is supplied separately to allow running the assay in the presence or absence of the IPC.

The use of a color compensation file generated with the Roche Diagnostics 'LightCycler®-Color Compensation Set' or with the Roche Diagnostics 'LightCycler® Multicolor Demo Set' is a prerequisite to run the duplex reaction.

The supplied standard row allows to determine the linear range of the reaction and to estimate the quantity of the target sequence in unknown samples.

For use in LightCycler® 1.x Instruments use channel F2 instead of channel 640 and channel F3 instead of channel 705 for detection.

3. Set contents

6 Vials with blue caps containing premixed lyophilized primers and probes for 16 PCR reactions each of *RVF Virus*
6 Vials with white caps containing the internal positive control (IPC)
1 Row with 6 lyophilized cloned plasmid standards of *RVF Virus* from 10^1 to 10^6 target equivalents per reaction
1 Sealing foil for the standard row

4. Programming

The protocol consists of four program steps

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Program:	**Denaturation**	**Cycling**			**Melting**			**Cooling**
Parameter								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	50			1			1
Segment	1	1	2	3	1	2	3	1
Target [℃]	95	95	55	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:10	00:00:08	00:00:15	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [℃/s]	20	20	20	20	20	20	0.2	20
Acquisition Mode	None	None	Single	None	None	None	Continu.	None

5. Data analysis

Switch the color compensation mode on. If this mode is not enabled run the color compensation program. Follow the instructions in the manual of the Roche Diagnostics 'LightCycler® – Color Compensation Kit'/ 'LightCycler® Multicolor Compensation Set'.

Perform data analysis, as described in the LightCycler® Instrument operator's manual.
We recommend using the Second Derivative Maximum method (Automated (F'' max)). The cycle number of the Crossing Point (CP) of each sample is calculated automatically. The Fit Points method is more-error prone due to the user's influence.

View *RVF Virus* data in channel 640, Quantification mode. The negative control (NTC) should show no signal. For the identification of the PCR product view *RVF Virus* data in channel 640, Melting Curves mode.

If the internal positive control is used, view data in channel 640, Quantification mode, and the IPC in channel 705, Quantification mode. The negative control and the low-concentrated *RVF Virus* DNA samples (10 to 1,000 copies) should show an amplification curve for the IPC with a CP at approximately cycle 30.

The provided standard row of cloned and purified DNA with concentrations in the range from 10^6 copies/rxn to 10^1 copies/rxn of *RVF Virus* should have CPs between cycles 18 and 35.

6. Product characteristics

PCR results are obtained within 1 hour.

Sensitivity
These reagents detect 10 copies of *RVF Virus* DNA (in an exemplary system, using cloned targets as reference).

Measuring range
The linear measuring range of the assay is 10^2 to 10^6 copies of *RVF Virus* DNA.

Storage and Stability
- Lyophilized reagents are stable for at least 3 months after shipment if stored protected from light at room temperature (18-25℃).
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 5 days if stored protected from light and refrigerated (4℃).

7. Experimental protocol

The following procedure was developed for use with the LightCycler® 1.x / 2.0 Instruments.
Start programming before preparing the solutions. See the LightCycler® Instrument operator's manual for details.

Sample material: Use aqueous nucleic acid preparations (e.g. Roche Diagnostics 'High Pure Viral Nucleic Acid Kit' combined with Roche Diagnostics 'Transcriptor First Strand cDNA Synthesis Kit').

Negative control: Always run at least one negative control - replace the template DNA with water.

Positive control: Run a positive control - replace the template DNA with the provided control DNA.
For quantification always use a fresh solution prepared from the provided standard row (single use only).

7.1. Preparation of parameter-specific reagents and reagents for the IPC (16 reactions):

One reagent vial with a **blue** cap contains all primers and probes to run 16 LightCycler® reactions for *RVF Virus*.
One reagent vial with a **white** cap contains all primers, probes and DNA to run 16 LightCycler® reactions for the IPC.

Add 66 µl PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

►Use 4 µl **reagent** for a 20 µl PCR reaction.

This solution is stable at least five days when stored refrigerated at 4°C. Avoid prolonged exposure t o light.

7.2. Preparation of the standard row

The target DNA is provided in 6 different quantities to yield from 10^1 to 10^6 target molecules in 5 µl once resuspended. Start with the lowest concentrated standard (first tube from the extended lip). Use the pipette tip to punch a hole through the sealing foil. Add 40 µl PCR-grade water to each vial of the row. Mix the target DNA by pipetting the solution up and down 10 times.

►Use 5 µl standard for a 20 µl PCR reaction.

This standard solution is not long-term stable and will lose sensitivity under prolonged storage. Use only fresh prepared solutions as quantification references. Older solutions can be used as a qualitative standard (positive control).
After adding the target DNA to the LightCycler® reaction mix use the provided sealing foil to close the vials in order to avoid changes in the concentration due to evaporation.
Please note that opening of these vials may cause contaminations of the work-space (aerosol).

7.3. Preparation of the LightCycler® reaction mix

In a cooled reaction tube, prepare the reaction mix by multiplying each volume for a single reaction by the number of reactions to be cycled plus one additional reaction.

For use with the Roche FastStart Master	
Single reaction	**Component**
3.4 µl	water, PCR-grade (colorless cap, provided with the Roche Master kit)
1.6 µl	Mg^{2+} solution 25 mM (blue cap, provided with the Roche FastStart kit)
4.0 µl	**reagent** mix (parameter specific reagents containing primers and probes, see **7.1.**)
4.0 µl	**IPC** mix (IPC reagents containing primers, probes and DNA, see **7.1.**)
2.0 µl	Roche Master (red cap, for preparation see Roche manual)
15.0 µl	**Volume of reaction mix**

To include the internal positive control add 4 µl of the IPC reagent per reaction to the reaction mix.
To run the assay without the internal positive control substitute the 4 µl of IPC with 4 µl PCR-grade water.

Mix gently, spin down and transfer 15 µl each of the reaction mix to a LightCycler® capillary.

Add 5 µl of sample or control DNA to each capillary for a final reaction volume of **20 µl.**

Start run.

8. Sample data - typical results

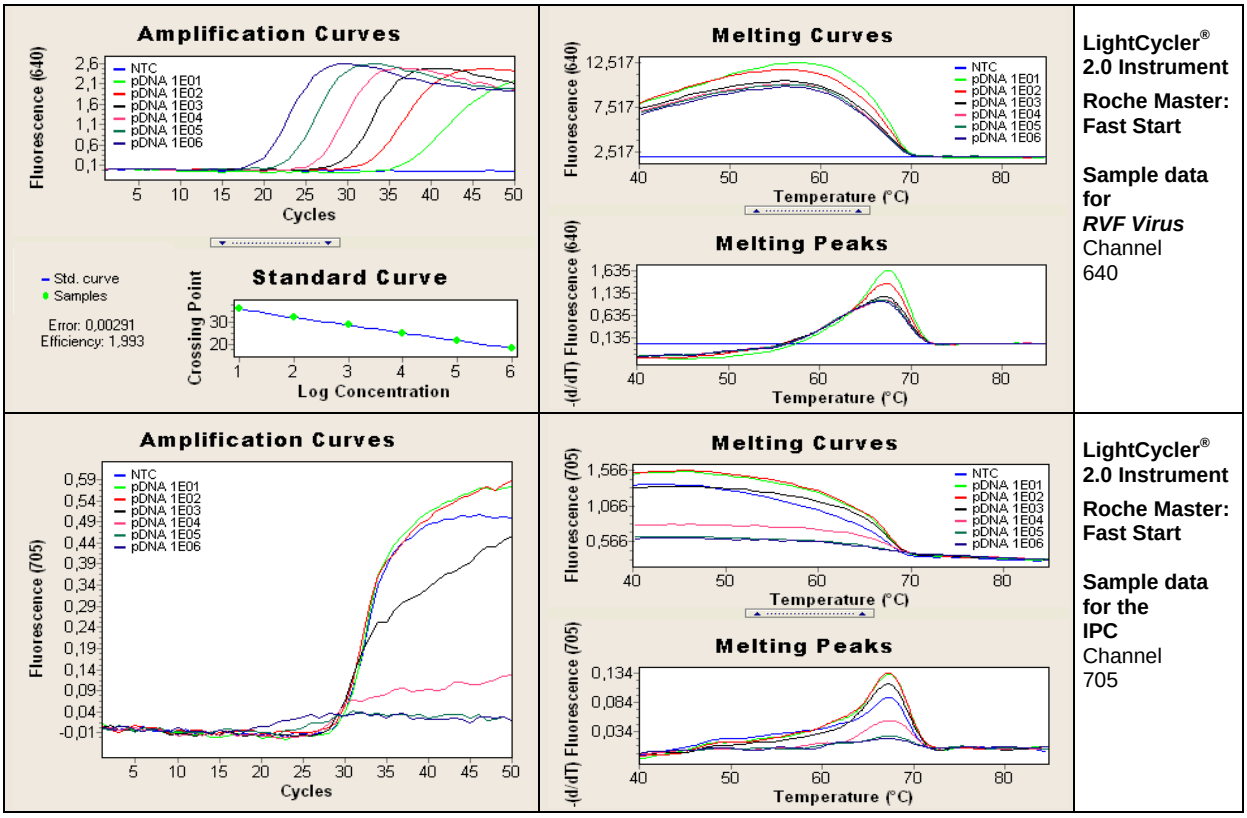

Fig.1. Sample data for the *RVF Virus* detection system.

Upper panels: Data from LightCycler® 2.0 Instrument. Left panel channel 640 quantification mode (Second Derivative Maximum) with calibration curve for *RVF Virus*. Right panel channel 640 melting analysis for *RVF Virus*.
Lower panels: Data from LightCycler® 2.0 Instrument. Left panel channel 705 quantification mode (Second Derivative Maximum) for the IPC. Right panel channel 705 melting analysis for the IPC.

9. Interpretation of data

Result	*RVF Virus* (sample)	IPC (sample)	NTC
Negative	no amplification	detectable	negative
Positive	amplification signal	not relevant	negative
PCR failure, repeat experiment	no amplification	not detectable	not relevant
Contamination, repeat experiment	amplification signal	not relevant	positive

Tab. 3. Typical analysis results (LightCycler® 2.0 Instrument, Roche Master: Fast Start)

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These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany.
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