# LightMix® Kit Respiratory Syncitial Virus (RSV)

**Cat.-No. 40-0115-32**

Kit with reagents for the detection of *RSV* DNA using the Roche Diagnostics LightCycler® 1.x / 2.0 / 480 II Instruments.

Lyophilized mix of primers and probes 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 N OT freeze!**

Instructions for use with the LightCycler® 1.x / 2.0 Instruments see pages 4-5
Instructions for use with the LightCycler® 480 II Instrument see pages 6-7

## 1. Introduction

Human Respiratory Syncytial Virus (RSV) is a negative ssRNA virus of the Paramyxoviridae family. RSV causes respiratory tract infections and is the most common cause for pneumonia and bronchiolitis in children under one year; by age two most children have been infected. The infection is very similar to the common cold and mild in adults and older healthy children. RSV infections can be severe in premature babies or infants with underlying health conditions, and in adults with lung and heart diseases and immune compromised individuals. In these cases an infection may require hospitalization. The virus spreads by aerosol and touch. Infected persons are most contagious a few days after infection; however the virus has been shown to spread a few weeks after the initial infection. Although RSV induces a protective immunity, the immunity decreases over time - maybe due to virus variability - making multiple infections possible.

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

This LightMix® Kit is tested on the LightCycler® 1.x / 2.0 / 480 II (96 well and 384 well formats) Instruments with Roche Diagnostics 'LightCycler® FastStart DNA Master HybProbe'.
A 1-step RT PCR procedure was not tested.

## 2. Description

A 232 bp fragment of *RSV* is amplified with specific primers. The resulting PCR fragment is analyzed with hybridization probes labeled with LightCycler® Red 640. The PCR product is identified by running a melting curve with a specific melting point at Tm 66.7°C.

The PCR reaction is monitored by an additional PCR product of 139 bp, formed from the internal control. This control does not interfere with the *RSV* specific reactions. The amplification will usually fail in the presence of higher concentrated *RSV* DNA samples (1,000 copies or higher) while displaying an amplification signal in negative and low-concentrated samples. The hybridization probes are labeled with LightCycler® Red 690. The IC is supplied separately to allow running the assay in the presence or absence of the IC.

The use of a color compensation file generated with the TIB MOLBIOL 'LightMix® Kit - Color Compensation 530/640/690' or the Roche Diagnostics 'LightCycler®-Color Compensation 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.

## 3. Set contents

3 Vials with blue caps containing premixed lyophilized primers and probes for 32 PCR reactions each of *RSV*
3 Vials with white caps containing the IC for 32 PCR reactions
1 Standard row with 6 lyophilized cloned plasmid standards of *RSV* from $10^1$ to $10^6$ target equivalents per reaction
1 Sealing foil for the standard row

## 4. Additional reagents and items required

*TIB MOLBIOL:*

| | |
|---|---|
| LightMix® Kit – Color Compensation 530/640/690 | Cat.-No. 40-0318-00 |

*Roche Diagnostics:*

| | |
|---|---|
| LightCycler® FastStart DNA Master HybProbe | Cat.-No. 03 003 248 001 |

*Roche Diagnostics optional items:*

| | |
|---|---|
| LightCycler® Multicolor Demo Set | Cat.-No. 03 624 854 001 |
| or LightCycler® Color Compensation Set (LightCycler® 1.x Instrument) | Cat.-No. 12 158 850 001 |
| High Pure RNA Isolation Kit | Cat.-No. 11 828 665 001 |
| Transcriptor First Strand cDNA Synthesis Kit | Cat.-No. 04 379 012 001 |
| LightCycler® Capillaries (20 µl) (LightCycler® 1.x / 2.0 Instruments) | Cat.-No. 04 929 292 001 |
| LightCycler® 480 Multiwell Plate 384, white (LightCycler® 480 Instrument) | Cat.-No. 04 729 749 001 |
| or LightCycler® 480 Multiwell Plate 96, white (LightCycler® 480 Instrument) | Cat.-No. 04 729 692 001 |

## 5. Product characteristics

PCR results are obtained within 45 minutes (50 cycles and melting curve) with the LightCycler® 1.x / 2.0 Instruments and within 80 minutes (50 cycles and melting curve) with the LightCycler® 480 II Instrument.

**Sensitivity**
These reagents detect 10 copies of *RSV* DNA using the Roche 'LightCycler® FastStart DNA Master HybProbe' with the LightCycler® 1.x / 2.0 / 480 II Instruments (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 *RSV* DNA using the Roche 'LightCycler® FastStart DNA Master HybProbe' with the LightCycler® 1.x / 2.0 / 480 II Instruments.

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

## 6. Experimental protocol

The following procedure was developed for use with the LightCycler® 1.x / 2.0 / 480 II 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 RNA Isolation Kit' combined with Roche Diagnostics 'Transcriptor First Strand cDNA Synthesis Kit').

**Negative control:** Always run at least one no-template control (NTC) - 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).

### 6.1. Preparation of parameter-specific reagents (32 reactions):

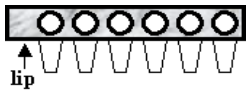
One reagent vial with a **blue** cap contains primers and probes to run 32 LightCycler® reactions for *RSV.*
One reagent vial with a **white** cap contains primers, probes and DNA to run 32 reactions for the IC.

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

►**Use 2 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.

### 6.2. 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 dissolved. 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 these vials may cause contaminations of the work-space (aerosol).

### 6.3. 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** |
| 6.6 µl | water, PCR-grade (colorless cap, provided with the Roche Master kit) |
| 2.4 µl | $Mg^{2+}$ solution 25 mM (blue cap, provided with the Roche FastStart kit) |
| 2.0 µl | **reagent** mix (parameter specific reagents containing primers and probes, see **6.1.**) |
| 2.0 µl | **IC** mix (IC reagents containing primers, probes and DNA, see **6.1.**) |
| 2.0 µl | Roche Master (red cap, for preparation see Roche manual) |
| **15.0 µl** | Volume of reaction mix |

Mix gently, spin down and **transfer 15 µl** each of the reaction mix to a LightCycler® capillary (LightCycler® 1.x / 2.0 Instrument) or to a multiwell plate (LightCycler® 480 II Instrument).

To include the internal control **add 2 µl** of the IC reagent per reaction to the reaction (as described).
To run the assay in the absence of the control substitute the 2 µl of IC with 2 µl PCR-grade water.

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

Start run.

# 7. LightCycler® 1.x / 2.0 Instruments

## 7.1. 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 to identify the PCR product as derived from the target DNA
- 4: Cooling: cooling the instrument

| Program Step: | Denaturation | Cycling | | | Melting | | | Cooling |
|---|---|---|---|---|---|---|---|---|
| **Parameter** | | | | | | | | |
| Analysis Mode | None | Quantification mode | | | Melting Curves mode | | | None |
| Cycles | 1 | 50 | | | 1 | | | 1 |
| Target [°C] | 95 | 95 | 62 | 72 | 95 | 40 | 85 | 40 |
| Hold [hh:mm:ss] | 00:10:00 | 00:00:05 | 00:00:05 | 00:00:15 | 00:00:20 | 00:00:20 | 00:00:00 | 00:00:30 |
| Ramp Rate [°C/s] | 20 | 20 | 20 | 20 | 20 | 20 | 0.2 | 20 |
| Sec Target [°C] | - | - | 55 | - | - | - | - | - |
| Step Size [°C] | - | - | 0.5 | - | - | - | - | - |
| Step Delay (Cycles) | - | - | 1 | - | - | - | - | - |
| Acquisition Mode | None | None | Single | None | None | None | Cont | None |

(Melting not relevant for detection)

## 7.2. Data Analysis

For use in LightCycler® 1.x Instruments with software version 3.5.3 read channel F2 instead of channel 640, channel F3 instead of channel 705 and channel F1 instead of channel 530 for detection. We recommend upgrading LightCycler® 1.x instruments to software version 4.1.

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 TIB MOLBIOL 'LightMix® Kit – Color Compensation 530/640/690 or the Roche Diagnostics 'LightCycler® – Color Compensation Kit' (LightCycler® 1.x Instruments) / 'LightCycler® Multicolor Demo Set' (LightCycler® 2.0 Instrument) .

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

If the internal control (IC) is used, view IC data in channel 705, Quantification mode. The negative control and the low-concentrated *RSV* DNA samples (10 to 1,000 copies) should show an amplification curve for the IC with a CP at approximately cycle 32.

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 *RSV* should have CPs between cycles 19 and 37.

## 7.3. Sample Data – typical results

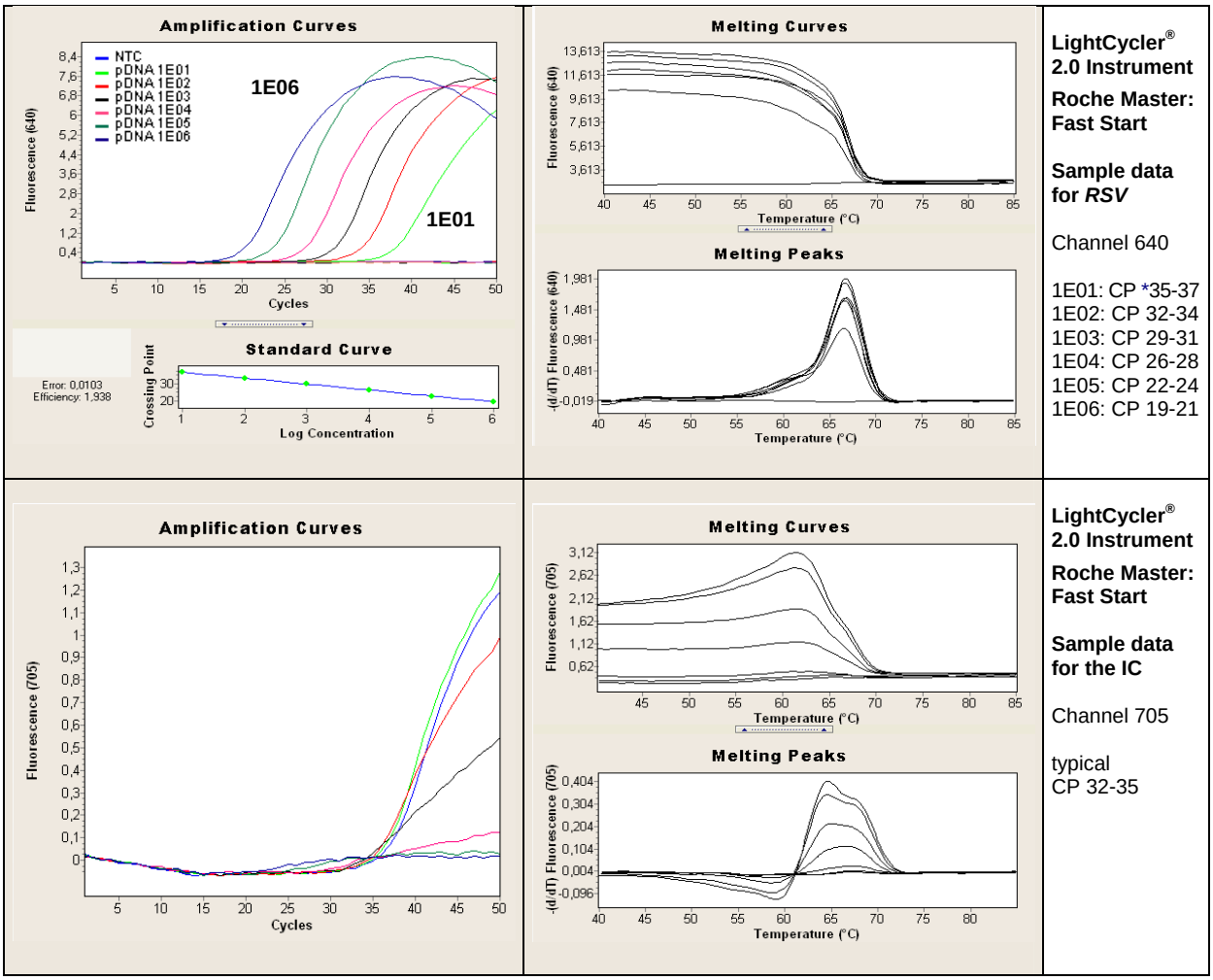



**Fig.1. Sample data for the *RSV* detection system.**

**Upper panels**: Data from LightCycler® 2.0 Instrument. Left panel channel 640 quantification mode (Second Derivative Maximum) with amplification curves for *RSV*. Right panel channel 640 melting analysis for *RSV* (not relevant for detection).

**Lower panels:** Data from LightCycler® 2.0 Instrument. Left panel channel 705 quantification mode (Second Derivative Maximum) for the IC. Right panel channel 705 melting analysis for the IC (not relevant for detection).

* **Note:** Fluorescence levels depend on instrument settings and may vary. The characteristics of the curve (shape and trend of fluorescence levels) should be similar to the curve in the manual. The fluorescent curves over cycles (quantification mode) must be smooth and not zigzag shaped. The CP values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (delta CP). The CP values described in this manual (chart text) have been obtained using the supplied standard row; the entire set of curves can be horizontal shifted.

## 7.4. Interpretation of data

| *RSV* (sample) | IC (sample) | NTC | Result |
|---|---|---|---|
| no amplification | detectable | negative | Negative |
| amplification signal | not relevant | negative | Positive |
| no amplification | not detectable | not relevant | PCR failure, repeat experiment |
| amplification signal | not relevant | positive | Contamination, repeat experiment |

**Typical analysis results (LightCycler® 2.0 Instrument, Roche Master: Fast Start)**

# 8. LightCycler® 480 II Instrument

## 8.1. 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

Detection Format:
LightCycler® 480 II Instrument: 465-510, 498-640, 498-660

| Program Step: | Denaturation | Cycling | | | Melting | | | Cooling |
|---|---|---|---|---|---|---|---|---|
| **Parameter** | | | | | | | | |
| Analysis Mode | None | Quantification mode | | | Melting Curves mode | | | None |
| Cycles | 1 | 50 | | | 1 | | | 1 |
| Target [°C] | 95 | 95 | 62 | 72 | 95 | 40 | 85 | 40 |
| Hold [hh:mm:ss] | 00:10:00 | 00:00:05 | 00:00:05 | 00:00:15 | 00:00:30 | 00:02:00 | 00:00:00 | 00:00:30 |
| Ramp Rate [°C/s] **96** | 4.4 | 4.4 | 2.2 | 4.4 | 4.4 | 1.5 | - | 1.5 |
| Ramp Rate [°C/s] **384** | 4.6 | 4.6 | 2.4 | 4.6 | 4.6 | 2.0 | - | 2.0 |
| Sec Target [°C] | - | - | 55 | - | - | - | - | - |
| Step Size [°C] | - | - | 0.5 | - | - | - | - | - |
| Step Delay (Cycles) | - | - | 1 | - | - | - | - | - |
| Acquisition Mode | None | None | Single | None | None | None | Continuous | None |
| Acquisitions [per °C] | - | - | - | - | - | - | 1* | - |

(Melting not relevant for detection)

## 8.2. 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 TIB MOLBIOL 'LightMix® Kit – Color Compensation 530/640/690' or the Roche Diagnostics 'LightCycler® Multicolor Demo 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 *RSV* data with Filter Combination 498-640 Quantification mode. The negative control (NTC) must show no signal. For the identification of the PCR product view *RSV* data with Filter Combination 498-640, Melting Curves mode.

If the internal control is used, view data with Filter Combination 498-640, Quantification mode, and the IC with Filter Combination 498-660, Quantification mode. The negative control and the low-concentrated *RSV* DNA samples (10 to 1,000 copies) should show an amplification curve for the IC with a CP at approximately cycle 32.

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 *RSV* should have CPs between cycles 19 and 37.

## 8.3. Sample Data – typical results

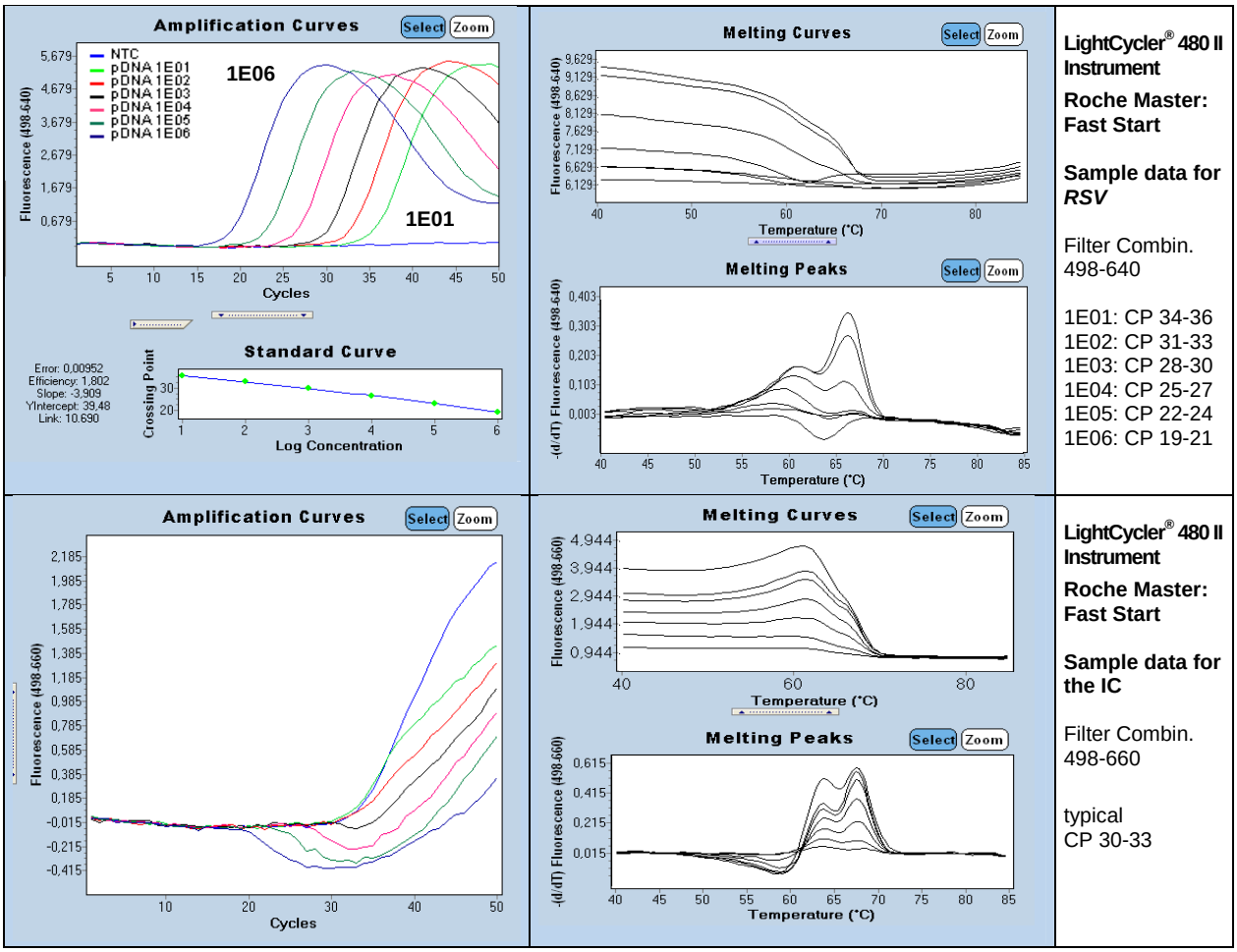



**Fig.2. Sample data for the *RSV* detection system.**

**Upper panels**: Data from LightCycler® 480 II Instrument. Left panel Filter Combination 498-640 quantification mode (Second Derivative Maximum) with amplification curves for RSV. Right panel Filter Combination 498-640 melting analysis for RSV (not relevant for detection).
**Lower panels**: Data from LightCycler® 480 II Instrument. Left panel Filter Combination 498-660 quantification mode (Second Derivative Maximum) with amplification curves for the IC. Right panel Filter Combination 498-660 melting analysis for the IC (not relevant for detection).

* **Note:** Fluorescence levels depend on instrument settings and may vary. The characteristics of the curve (shape and trend of fluorescence levels) should be similar to the curve in the manual. The fluorescent curves over cycles (quantification mode) must be smooth and not zigzag shaped. The CP values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (delta CP). The CP values described in this manual (chart text) have been obtained using the supplied standard row; the entire set of curves can be horizontal shifted.

## 8.4. Interpretation of data

| ***RSV*** **(sample)** | **IC (sample)** | **NTC** | **Result** |
|---|---|---|---|
| no amplification | detectable | negative | Negative |
| amplification signal | not relevant | negative | Positive |
| no amplification | not detectable | not relevant | PCR failure, repeat |
| amplification signal | not relevant | positive | Contamination, repeat |

**Typical analysis results (LightCycler® 480 II Instrument, Roche Master: Fast Start)**

## 9. Version history

| | |
|---|---|
| V_110223 | 32 rxn per vial |
| V_111102 | Correction of IC bp size |

**Notice to Purchaser**

A license under U.S. Patents 4,683,202, 4,683,195 and 4,965,188 or their foreign counterparts, owned by Hoffmann-La Roche Inc. and F. Hoffmann-La Roche Ltd (“Roche”), has an up-front fee component and a running-royalty component. The purchase price of this product includes limited, nontransferable rights under the running-royalty component to use only this amount of the product to practice the Polymerase Chain Reaction (“PCR”) and related processes described in said patents solely for the research and development activities of the purchaser when this product is used in conjunction with a thermal cycler whose use is covered by the up-front fee component. Rights to the up-front fee component must be obtained by the end user in order to have a complete license. These rights under the upfront fee component may be purchased from Perkin-Elmer or obtained by purchasing an authorized thermal cycler. No right to perform or offer commercial services of any kind using PCR, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is hereby granted by implication or estoppel. Further information on purchasing licenses to practice the PCR process for research applications may be obtained by contacting the Director of Licensing at The Perkin-Elmer Corporation, 850 Lincoln Center Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501. The purchase of this product does not convey any right for its use in clinical diagnostic applications. No rights for TaqMan technology under U.S. Patents 5,210,015 and 5,487,972 are hereby conveyed.

These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin Germany.
This LightMix® Kit sold under license from Roche Diagnostics GmbH.

4260159332209