

## LightMix<sup>®</sup> Kit *Klebsiella* KPC

Cat.-No. 40-0598-32

Kit with reagents for the detection of *Klebsiella Pneumoniae* Carbapenemases (KPC) DNA using the Roche Diagnostics LightCycler<sup>®</sup> 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 NOT freeze!**

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

### 1. Introduction

*Klebsiella pneumoniae* carbapenemases (KPC) is a multi-drug resistant bacteria first discovered 2001 in the USA. The KPC enzymes that inactivate all penicillins, cephalosporins, aztreonam and carbapenems are encoded on plasmids that are shared among bacteria. KPC resistance has spread to many countries and has been found in many different gram negative bacteria. Infections with KPC bacteria are in most cases nosocomial, tend to occur in patients with multiple co-morbidities and have a very high mortality rate. Rapid detection and identification of KPC strains is important to ensure patient isolation and rapid treatment with adequate antibiotics.

The LightMix<sup>®</sup> Kit *Klebsiella* KPC 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<sup>®</sup> Kit is tested on the LightCycler<sup>®</sup> 1.x / 2.0 / 480 II Instrument (96 well and 384 well formats) Instruments with Roche Diagnostics 'LightCycler<sup>®</sup> FastStart DNA Master HybProbe'.

### 2. Description

A 101 bp fragment of the *Klebsiella* KPC DNA is amplified with specific primers. The resulting PCR fragment is analyzed with hybridization probes labeled with LightCycler<sup>®</sup> Red 640 (detected in channel 640).

The PCR reaction is monitored by an additional PCR product of 349 bp, formed from the internal control. This control does not interfere with the *Klebsiella* KPC specific reactions. The amplification will usually fail in the presence of higher concentrated *Klebsiella* KPC 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<sup>®</sup> Red 705 (recorded in channel 705). 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<sup>®</sup> Kit - Color Compensation 530/640/690' or the Roche Diagnostics 'LightCycler<sup>®</sup>-Color Compensation Set' or with the Roche Diagnostics 'LightCycler<sup>®</sup> Multicolor Demo Set' is a prerequisite to run the duplex reaction.

The supplied standard row DNA 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 green caps containing premixed lyophilized primers and probes for 32 PCR reactions each of *Klebsiella KPC3*
- 3 Vials with white caps containing the internal control (IC)
- 1 Standard row with 6 lyophilized cloned plasmid standards of *Klebsiella KPC* 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<sup>®</sup> Kit – Color Compensation 530/640/690 Cat.-No. 40-0318-00

#### Roche Diagnostics:

LightCycler<sup>®</sup> FastStart DNA Master HybProbe Cat.-No. 03 003 248 001

LightCycler<sup>®</sup> Multicolor Demo Set Cat.-No. 03 624 854 001

or LightCycler<sup>®</sup> Color Compensation Set (LightCycler<sup>®</sup> 1.x Instrument) Cat.-No. 12 158 850 001

High Pure PCR Template Preparation Kit Cat.-No. 11 796 828 001

LightCycler<sup>®</sup> Capillaries (20 µl) (LightCycler<sup>®</sup> 1.x / 2.0 Instruments) Cat.-No. 04 929 292 001

LightCycler<sup>®</sup> 480 Multiwell Plate 384, white (LightCycler<sup>®</sup> 480 Instrument) Cat.-No. 04 729 749 001

or LightCycler<sup>®</sup> 480 Multiwell Plate 96, white (LightCycler<sup>®</sup> 480 Instrument) Cat.-No. 04 729 692 001

### 5. Product characteristics

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

#### Sensitivity

These reagents detect 10 copies of *Klebsiella KPC* DNA using the Roche 'LightCycler<sup>®</sup> FastStart DNA Master HybProbe' with the LightCycler<sup>®</sup> 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 *Klebsiella KPC* DNA using the Roche 'LightCycler<sup>®</sup> FastStart DNA Master HybProbe' with the LightCycler<sup>®</sup> 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 PCR Template Preparation 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 and reagents for the IC (32 reactions):

One reagent vial with a **green** cap contains primers and probes to run 32 LightCycler® reactions for *Klebsiella KPC*.

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

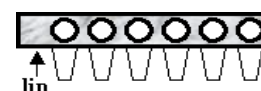
**Add 66 µl** PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

► **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 to 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
7.0 µl	water, PCR-grade (colorless cap, provided with the Roche Master kit)
2.0 µl	Mg <sup>2+</sup> solution 25 mM (blue cap, provided with the Roche FastStart kit)
2.0 µl	<b>reagent</b> mix (parameter specific reagents containing primers and probes, see 6.1.)
2.0 µl	<b>IC</b> 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

To include the internal control **add 2 µl** of the IC reagent per reaction to the reaction mix.

To run the assay without the internal control substitute the 2 µl of IC with 2 µl PCR-grade water.

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).

**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 for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Program Step:	Denaturation	Cycling			Melting			Cooling
<b>Parameter</b>								
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 select channel F2 instead of channel 640, channel F3 instead of channel 705 for detection.

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 Instrument) or the 'LightCycler® Multicolor Demo Set' (LightCycler® 2.0 Instrument).

Perform data analysis, as described in the LightCycler® Instrument operator's manual.

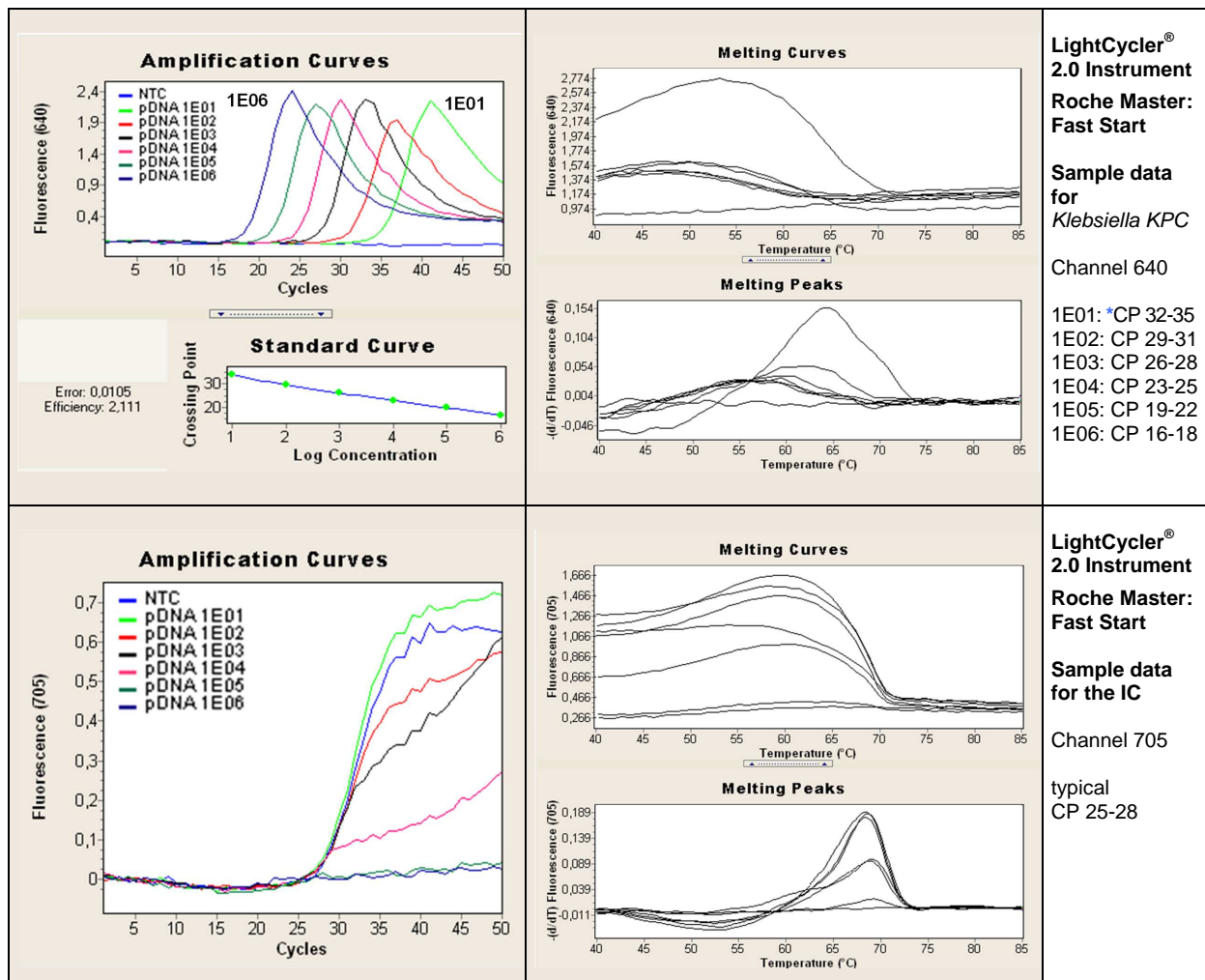
We recommend using the Second Derivative Maximum method (Automated (F" max)). The cycle number of the Crossing Point (CP) of each sample is calculated automatically. The Fit Points method is more-error prone due to the user's influence.

View *Klebsiella KPC* data in channel 640, Quantification mode. The negative control (NTC) must show no signal.

If the internal control (IC) is used, view IC data in channel 705, Quantification mode. The negative control and the low-concentrated *Klebsiella KPC* DNA samples (10 to 1,000 copies) should show an amplification curve for the IC with a CP at approximately cycle 27.

The provided standard row of cloned and purified DNA with concentrations in the range from 10<sup>6</sup> copies/rxn to 10<sup>1</sup> copies/rxn of *Klebsiella KPC* should have CPs between cycles 16 and 35.

### 7.3. Sample Data – typical results



**Fig.1. Sample data for the *Klebsiella KPC* detection system.**

**Upper panels:** Data from LightCycler® 2.0 Instrument. Left panel channel 640 quantification mode (Second Derivative Maximum) with amplification curves for *Klebsiella KPC*. Right panel channel 640 melting analysis for *Klebsiella KPC* (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

Klebsiella KPC (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.

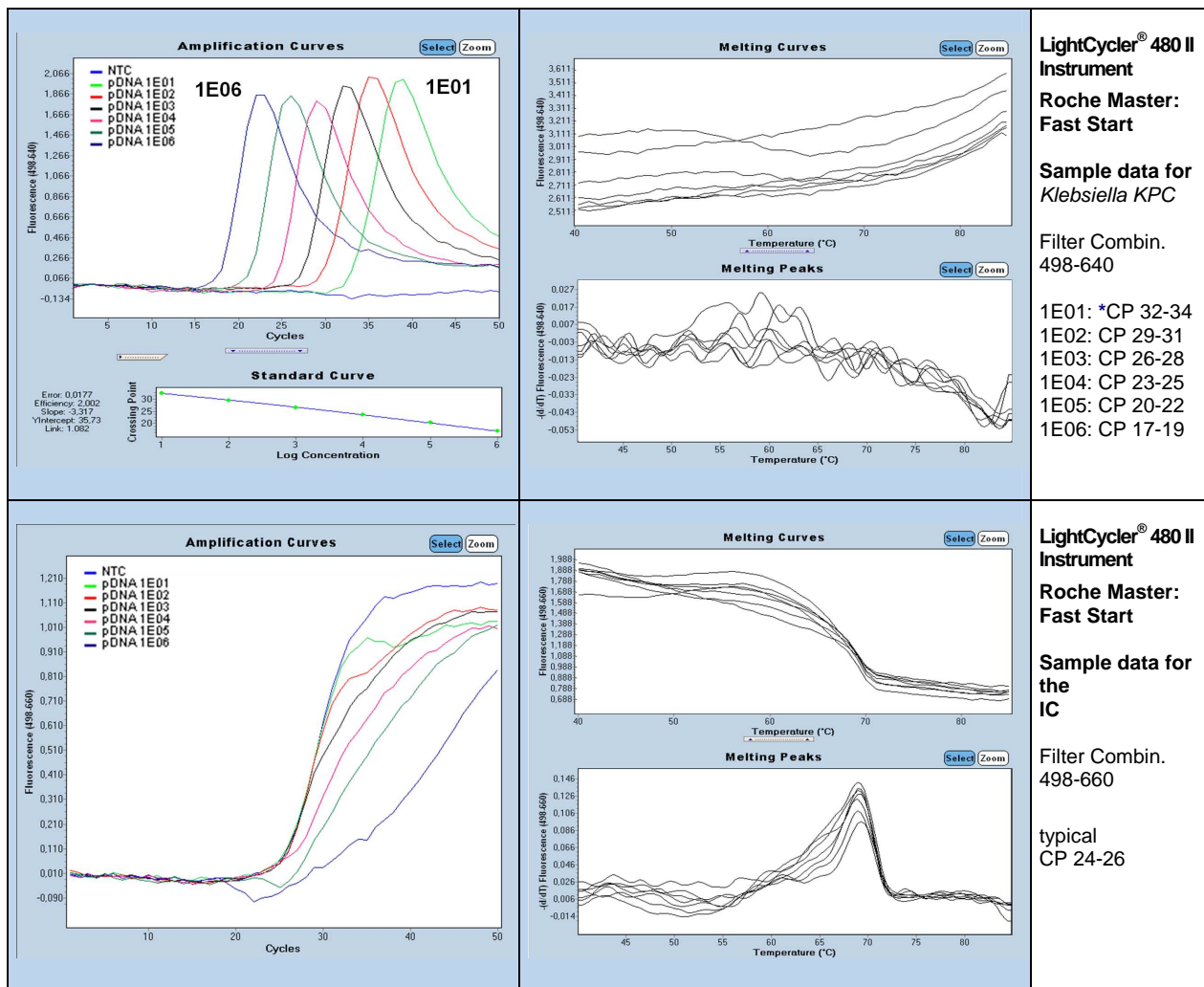
We recommend using the Second Derivative Maximum method (Automated (F" max)). The cycle number of the Crossing Point (CP) of each sample is calculated automatically. The Fit Points method is more-error prone due to the user's influence.

View *Klebsiella KPC* data with Filter Combination 498-640, Quantification mode. The negative control (NTC) must show no signal.

If the internal control is used, view *Klebsiella KPC* data with Filter Combination 483-640, Quantification mode, and the IC data with Filter Combination 498-660, Quantification mode. The negative control and the low-concentrated *Klebsiella KPC* DNA samples (10 to 1,000 copies) should show an amplification curve for the IC with a CP at approximately cycle 25.

The provided standard row of cloned and purified DNA with concentrations in the range from 10<sup>6</sup> copies/rxn to 10<sup>1</sup> copies/rxn of *Klebsiella KPC* should have CPs between cycles 17 and 34.

### 8.3. Sample Data – typical results



**Fig.1. Sample data for the *Klebsiella KPC* 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 *Klebsiella KPC*. Right panel Filter Combination 498-640 melting analysis for *Klebsiella KPC* (not relevant for detection).

**Lower panels:** Data from LightCycler® 480 II Instrument. Left panel Filter Combination 498-660 quantification mode (Second Derivative Maximum) with melting analysis 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

Klebsiella KPC (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)**

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