

8. Sample data - typical results

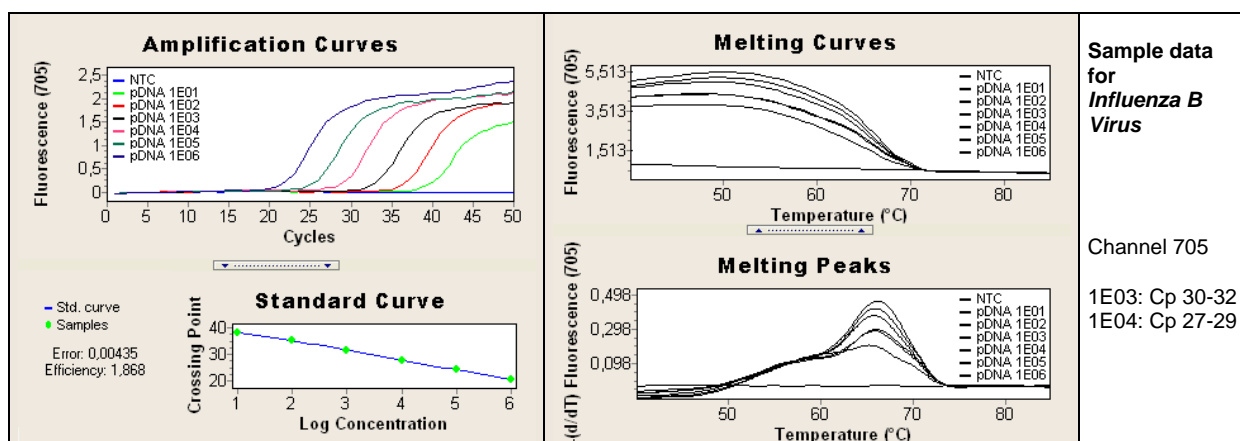


Fig.1. Sample data for the *Influenza B Virus* detection system.

Data from channel 705. Left panel quantification (Second Derivative Maximum) with calibration curve. Right panel melting curves for the target (not relevant for detection, shape varies with concentration).

9. Interpretation of data

Influenza B Virus (sample)	Negative Control NTC	Result
No amplification	Negative	Negative (Not detectable)
Amplification Cp < 36	Negative	Positive
Amplification signal	Positive	Contamination, repeat experiment

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

10. Material Safety Data

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and the EU Directives 67/548/EC and 1999/45/EC any products which not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Material Safety Data Sheet (MSDS).

Product is not hazardous, not toxic, not IATA-restricted. Product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

11. Version history

V060510 Release version
V130705 MSDS included, Editorial changes

Roche SAP order n° 05552389001

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These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany.
LightCycler® hybridization probes produced under license from Roche Diagnostics.



LightMix[®] Kit *Influenza B Virus* Cat.-No. 40-0225-16

Reagents for the detection of *Influenza B Virus* cDNA using the LightCycler[®] Instrument 1.x / 2.0.

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!

Additional reagents required (Roche Diagnostics):

LightCycler [®] FastStart DNA Master ^{PLUS} HybProbe	Cat.-No. 03 515 575 001
or LightCycler [®] FastStart DNA Master HybProbe	Cat.-No. 03 003 248 001
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

Influenza B Virus, a positive strand RNA virus, causes respiratory Influenza primarily in humans with several symptoms like a dry, hacking cough, sore throat, headache and limb pains. Nasal discharge and sneezing are common.

The antigenic variation of *Influenza B Virus* is less extensive than in type A viruses: no distinct subtypes or variants of hemagglutinin and neuraminidase are recognized.

Epidemics are less likely than with *Influenza A virus* and there have been no pandemics.

Influenza B Virus has been isolated from seals which may constitute the animal reservoir from which humans are exposed.

The LightMix[®] Kit for the detection of cDNA from *Influenza B Virus* provides a fast, easy and accurate system to identify and quantify this virus.

This LightMix[®] Kit is tested with the Roche Diagnostics "LightCycler[®] FastStart DNA Master Hybridization Probes" ready-to-use reaction mix in the LightCycler[®] Instrument 2.0.

2. Description

This LightMix[®] detects a part of the *Influenza B Virus* genome indicating the presence of *Influenza B Virus* cDNA in a nucleic acid extract.

A 184 bp fragment of the *Influenza B Virus* genome is amplified with specific primers and detected with probes labeled with LightCycler[®] Red 705 (detected in channel 705). The PCR product is identified by running a melting curve with a melting point (T_m) of 65,5°C in channel 705.

The supplied standard row allows the absolute quantification of the unknown samples.

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.

3. Set contents

- 6 Vials with blue cap containing lyophilized primers and hybridization probes for 16 reactions each
- 1 Row with 6 lyophilized cloned standards of *InfB* 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

- Program 1: Denaturation of sample and activation of the enzyme
- Program 2: PCR-amplification of the target DNA
- Program 3: Melting curve for identification of the *Influenza B Virus* DNA derived PCR product
- Program 4: 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	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:05	00:00:15	00:00:05	00:00:20	00:00:30	00:00:00	00:00:30
Ramp Rate [°C/s]	20	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	None	Cont	None

(Melting not relevant for detection)

Note: Universal step-down cycling program. No changes in reagents made. The former cycling program yields equivalent results.

5. Data analysis

Perform data analysis, as described in the LightCycler® 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 user's influences.

View *Influenza B Virus* data in channel 705, Quantification mode. The negative control (NTC) must show no signal. For the identification of the PCR product view *Influenza B Virus* data in channel 705, Melting Curves mode.

Typical results (Software Version 4.0)

The provided standard row of cloned and purified DNA with concentrations in the range from 10⁶ copies/rxn to 10¹ copies/rxn should have Cp values between cycles 18 and 35 (Cp values calculated with Second Derivative Maximum method).

6. Product characteristics

PCR results are obtained within 1 hour.

Sensitivity

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

Measuring range

The linear measuring range of the assay is 10² to 10⁶ copies of *Influenza B Virus* DNA.

Storage and Stability

- Lyophilized reagents are stable for at least 6 months after shipment when stored protected from light at room temperature (18-25°C). See expiry date on the product label.
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 10 days when stored protected from light and refrigerated (4°C).
- Dissolved reagents can be long-term stored frozen at -20°C. Avoid multiple thaw-freeze cycles.

7. Experimental protocol

The following procedure was developed for use with the LightCycler® Instrument 1.x / 2.0. Start programming before preparing the solutions. See the 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 (16 reactions):

One reagent vial with a **blue** cap contains all primers and probes to run 16 reactions for *InfB*.

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 for three days or longer if stored refrigerated at 4°C. Avoid prolonged exposure to 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 resolved. 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 reaction tube cooled below 4°C, 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 ^{PLUS} kit		For use with the Roche FastStart kit	
Single reaction	Component	Single reaction	Single reaction
7.0 µl	water, PCR-grade (colorless cap, provided with the Roche FastStart or FastStart ^{PLUS} kit)	7.4 µl	
--	Mg ²⁺ solution 25 mM (blue cap, provided with the Roche FastStart kit)	1.6 µl	
4.0 µl	reagent mix (parameter specific reagents containing primers and probes, see A)	4.0 µl	
4.0 µl	FastStart mix (vial 1 (red cap), combined from vials 1a and 1b, see Roche manual)	2.0 µl	
15.0 µl		15.0 µl	
Volume of reaction mix			

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

Add 5 µl of sample or standard (standard dilutions of control target, see instruction **7.2**) to each capillary to give a final reaction volume of **20 µl**.

Start run.