LightMix® Kit *InfA InfB with Extraction Control*

Cat.-No. 40-0607-96

Kit with reagents for the detection of the *Influenza A and Influenza B genomic RNA or* cDNA using the Roche Diagnostics LightCycler® 480 / 480 II and Cobas® Z 480 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℃), do N OT freeze!**

1. Introduction

Influenza A and Influenza B are positive single-strand RNA viruses, affecting the respiratory system and causing Influenza. Typical symptoms are a dry, hacking cough, sore throat, headache and limb pains. Nasal discharge and sneezing are common.

All type A influenza viruses, including those that regularly cause seasonal epidemics of influenza in humans, are genetically variable and well adapted to elude host defenses. Influenza viruses can recombine due to the fact that they have seven separate genomic elements. They lack mechanisms for 'proofreading' and repair of errors occuring during replication and as a result of uncorrected errors the genetic composition of the viruses changes as they replicate in humans and animals.

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 irus has been isolated from seals which may constitute the animal reservoir from which humans are exposed.

The LightMix® Kit *Inf A Inf B Extraction Control* provides a fast, easy and accurate system to identify these targets in a nucleic acid extract. A control amplification reaction acts as internal control (IC).

This LightMix® Kit is tested on the LightCycler® 480 Instruments with Roche Diagnostics 'LightCycler® FastStart DNA Master HybProbe' and with Roche Diagnostics 'LightCycler® RTR RNA Virus Master'.

2. Description

A 99 bp fragment of the *Inf A Virus* genome and a 103 bp fragment of the *Inf B Virus* genome is amplified with specific primers. The resulting PCR fragments are analyzed with hydrolysis probes detected with filter combination 465-510 for Influenza A, and filter combination 533-580 for Inf B.

The kit includes an extraction control based on a fragment of the growth differentiation factor 8 gene (GDF-8) which is highly conserved in chordata, allowing to work with samples from many species. The control PCR reaction does not interfere with the virus specific reactions and generates a 89 bp fragment which is detected by a LC670 labelled probe, recorded in filter combination 618-660.
The control assay will fail in the presence of higher concentrated Influenza-positive samples (1,000 copies or higher) while displaying an amplification signal in negative and low-concentrated samples.

The instrument requires a color compensation generated with the Hexaplex Color Compensation Kit.

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

3. Set contents

1 Vial with blue cap containing premixed lyophilized primers / probes for 96 PCR reactions
1 Standard row with 6 lyophilized cloned standards *Inf A* from 10^1 to 10^6 target equivalents per rxn
1 Standard row with 6 lyophilized cloned standards *Inf B* from 10^1 to 10^6 target equivalents per rxn
2 Sealing foils for the standard rows

4. Additional reagents and items required

TIB MOLBIOL:
LightMix® Kit – Hexaplex Color Compensation Cat.-No. 40-0320-00

Roche Diagnostics:
LightCycler® FastStart DNA Master HybProbe Cat.-No. 03 003 248 001
RealTimeReady RNA Virus Master Cat.-No. 05 619 416 001
High Pure RNA Isolation Kit Cat.-No. 11 828 665 001
High Pure Viral Nucleic Acid Kit Cat.-No. 11 858 874 001
Transcriptor Reverse Transcriptase kit Cat.-No. 03 531 295 001
LightCycler® 480 Multiwell Plate 96, white (LightCycler® 480 Instruments) Cat.-No. 04 729 692 001

5. Product characteristics

PCR results are obtained within 80 minutes (50 cycles) with the LightCycler® 480 Instruments.

Sensitivity
This reagent detects 10 copies of cloned target using 'LightCycler® FastStart DNA Master HybProbe'.

Measuring range
The linear measuring range of the assay is 10^2 to 10^6 copies target (FastStart DNA Master HybProbe)

Storage and Stability
• Dry reagents are stable for at least 3 months (18-25℃). Please see expiry on the product label
• **Do not freeze** dry reagents. Dissolved reagents are stable for at least 5 days (in the dark at 4℃).

6. Instrument Programming

Detection Format:
LightCycler® 480 Instrument: 483-533, 523-568, 615-670
LightCycler® 480 II Instrument: 465-510, 533-580, 618-660
Cobas® Z 480 Instrument: 465-510, 540-580, 610-670

6.1. Programming for the use with RealTimeReady RNA Virus Master

The protocol consists of five program steps

• 1: Reverse Transcription (skip for use with FastStart)
• 2: Denaturation: sample denaturation and enzyme activation (extend to 600 s to use with FastStart)
• 3: Cycling: PCR-amplification of the target DNA
• 4: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
• 5: Cooling: cooling the instrument

Program Step:	RT	Denaturation	Cycling			Cooling
Parameter						
Analysis Mode	None	None	Quantification mode			None
Cycles	1	1	50			1
Target [℃]	58	95	95	62	72	40
Hold [hh:mm:ss]	00:08:00	00:00:30	00:00:05	00:00:05	00:00:15	00:00:30
Ramp Rate [℃/s] **96**	4.4	4.4	4.4	2.2	4.4	2.2
Ramp Rate [℃/s] **384**	4.6	4.6	4.6	2.4	4.6	2.0
Sec Target [℃]	-	-	-	55	-	-
Step Size [℃]	-	-	-	0.5	-	-
Step Delay (Cycles)	-	-	-	1	-	-
Acquisition Mode	None	None	None	Single	None	None
Acquisitions [per ℃]		-	-	-	-	-

6.2. Programming for the use with FastStart

Replace step 1 and step 2 by 600 sec denaturation / activation at 95℃.

7. Experimental protocol

The following procedure was developed for use on the LightCycler® 480 / 480II and Cobas® Z 480 Instruments.
Start programming before preparing the solutions. See the 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.

7.1. Preparation of parameter-specific reagents (96 reactions):

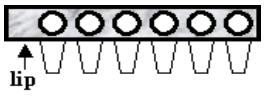
One reagent vial with a **blue** cap contains primers and probes to run 96 LightCycler® reactions.

Add 100 µl PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

►**Use 1 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.

7.2. Preparation of the standard rows



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 with 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 contamination 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 (cDNA)	
Single reaction	**Component**
9.6 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)
1.0 µl	**reagent** mix (parameter specific reagents containing primers and probes, see **6.1.**)
2.0 µl	Roche Master (red cap, for preparation see Roche manual)
15.0 µl	Volume of reaction mix

For use with the Roche RTR RNA Virus Master (RNA)	
Single reaction	**Component**
9.6 µl	water, PCR-grade (colorless cap, provided with the Roche Master kit)
4.0 µl	5 x Reaction Buffer RTR RNA Master
0.4 µl	50 x Enzyme Mix RTR RNA Master
1.0 µl	**reagent** mix (parameter specific reagents containing primers and probes,)
15.0 µl	Volume of reaction mix

Mix gently, spin down and **transfer 15 µl** each of the reaction mix to a multiwell plate.

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

Start run.

8. Data Analysis

8.1. Data Analysis

Switch the color compensation mode on. Perform data analysis as described in the operator's manual. We recommend using the Second Derivative Maximum method (Automated (F" max)).

View *Inf A* results in Filter Combination 465-510, Inf B in 533-580, and Extraction Control in 618-660, Quantification mode. The negative control (NTC) must show no signal.

The standard row covers a range from 10^6 to 10^1 copies/rxn with Cp values between cycles 22 and 35.

8.2. Typical Results

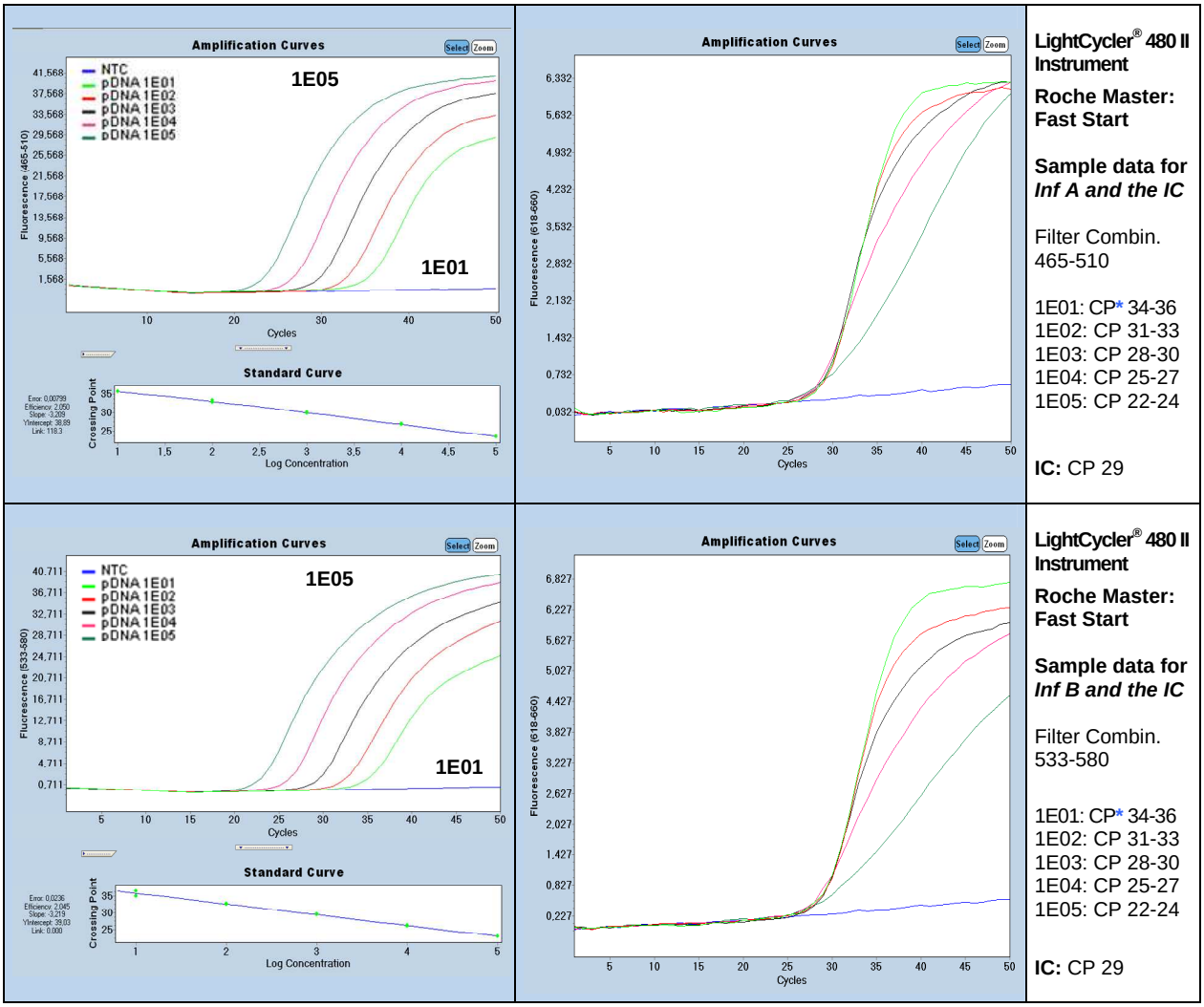


Fig.1. Sample data for the *Inf A Inf B* detection system. Data from LightCycler® 480 II Instrument.

8.3. Interpretation of data

Inf A **filter 510 (sample)**	***Inf B*** **filter 580 (sample)**	**Extraction Control (sample)**	**Negative Control (NTC)**	**Result InfA or InfB**
no amplification		detectable	negative	Negative
amplification signal		not relevant	negative	Positive
no amplification		not detectable	not relevant	Failure, repeat
not relevant		not relevant	positive	Contamination, repeat

Typical analysis results (LightCycler® 480 Instruments, Roche Master: Fast Start)

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