Instructions For Use

LightMix® Modular Ebola Virus Zaire (2014) 530

Cat.-No. 53-0649-96 Roche SAP n° 07 383 428 001

Kit with reagents for 96 PCR reactions 20 µl for detection of Ebola Zaire (2014) RNA [lyophilized]

1. Content, Storage and Expiry

1 Vial yellow cap 96 reactions Ebola (lyophilized)
1 Vial black cap Positive Control (RNA), Cp-value ~ 30

Storage at Arrival:

Store cooled or at ambient temperature
Do not freeze the lyophilized reagents.

- Lyophilized kits are stable for at least 6 months (4°C to 25°C in the dark). See lot-specific expiry date.
- Dissolved reagents are stable for at least 2 weeks if stored protected from light and cooled (4°C).
- Dissolved reagents can be stored long-term at -20°C (within expiry). Avoid multiple freeze-thaw cycles.
- Dissolved positive controls must be stored at -20°C. Avoid multiple freeze-thaw cycles.

2. Additional Reagents required

LightCycler® Multiplex RNA Virus Master Cat.-No. 06 754 155 001

3. Introduction

Ebola virus (EBOV), known since the first outbreak in Zaire (today Democratic Republic of Congo) in 1976, is a ssRNA Filovirus causing severe disease typically associated with hemorrhagic fever (EHF). Depending on the respective strain the mortality is reported to be between 20% up to more than 90%. The virus and speicmen must be handled under Level-4 Biosafety conditions. EBOV is listed as potential bioweapon, explaining why detection assays/kits are under EU report restrictions (dual-use-goods).

4. Description

A 127 bp long fragment from the viral RNA-Polymerase (L protein) gene is amplified with specific primers and detected with a FAM labeled hydrolysis probe (530 channel).

Notes : 1 -This kit can be applied with the Multiplex RNA master on LightCycler® 2.0 instruments.
2 -This kit can be combined with the Roche Diagnostics Realtime Ready RNA Virus master.
3 -The annealing temperature can be increased up to 64°C (instructions for 1-3 not included).

5. Specification

This assay detects 10 genome equivalent copies or less per reaction (in vitro transcribed RNA).

6. Sample Material and Extraction

Typical clinical samples are blood or serum; the virus can be detected also from fecal samples.
See ModularDx Document ***Extraction Protocols***.

7. Material Safety Data (MSDS)

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and the EU Directives 67/548/EC and 1999/45/EC any products which do 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.

8. Instructions for Use

• Instrument programming see document ***ModularDx Programming***
• Color Compensation see instructions in ***40-0320 Universal Color Compensation Hexaplex***
• Pipetting instructions multiplex PCR see ***ModularDx Multiplex***

8.1. Programming Roche 480 Instruments

See the Instrument operator's manual for details. Start programming before preparing the solutions. The protocol consists of four program steps:

• 1: Reverse Transcription of the viral RNA
• 2: Denaturation: sample denaturation and enzyme activation
• 3: Cycling: PCR-amplification
• 4: Cooling: cooling the instrument

Detection Format 530 Channel **Set Quant Factor 10, Max Integration time 1 sec**
LightCycler® 480 Instrument: 483-533
LightCycler® 480 II Instrument: 465-510
cobas z 480 Analyzer (open channel): 465-510

Program Step:	***RT Step***	***Denaturation***	***Cycling***			***Cooling***
Parameter						
Analysis Mode	***None***	None	Quantification mode			None
Cycles	1	1	45			1
Target [°C]	55	95	95	60	72	40
Hold [hh:mm:ss]	00:05:00	00:05:00	00:00:05	00:00:15	00:00:15	00:00:30
Ramp Rate [°C/s] **96**	4.4	4.4	4.4	2.2	4.4	1.5
Ramp Rate [°C/s] **384**	4.6	4.6	4.6	2.4	4.6	2.0
Acquisition Mode	None	None	None	**Single**	None	None

Table 1

8.2. Experimental Protocol

• **Sample material:** Use aqueous nucleic acid preparations (e.g. 'High Pure Viral Nucleic Acid Kit').
• **Negative control:** Always run at least one no-template control (NTC) - replace the template NA with water.
• **Positive control:** Run a positive control - replace the template NA with the provided Positive Control.

For an increased sensitivity use 10 µl sample per 20 µl reaction, in case that inhibition is likely to occur, e.g. extracts obtained from fecal samples, use 5 µl. For 10 µl reactions in 384 well plates use 5 µl /2.5 µl.

8.2.1. Preparation of Parameter-Specific Reagents (PSR, 96 reactions):

One reagent vial with a **yellow** cap contains all primers and probe to run 96+ LightCycler® reactions.

Add 50 µl PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.
For robotic pipetting the volume can be extended to 55 µl (signals will decrease by 10-20%).

►**Use 0.5 µl** reagent for a 20 µl PCR reaction.

8.2.2. Preparation of the Positive Control

Add 160 µl RNase/DNase-free Tris buffer or water to the vial with the **black** cap, for 10 µl sample add **320 µl**. Mix the solution by pipetting up and down 10 times. If vortexing spin down to collect the solution.

Notes: Opening of this vial may cause contaminations of the work-space (aerosol). Use of Tris buffer pH 8.0-8.5 increases the long-term stability in solution. Store dissolved controls frozen.

►**Use 5 µl** positive control (≈ 1,000 copies) for a 20 µl PCR reaction (or 10 µl if using 10 µl sample).

8.2.3. Preparation of the Reaction Mix

Multiply volumes by the number of reactions plus controls and one reserve and prepare in a cooled tube:

For use with the Roche LightCycler® Multiplex RNA Virus Master		
for 5 µl extract	**Component**	**10 µl extract**
10.4 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	5.4 µl
0.5 µl	**Reagent** mix (parameter specific reagents containing primers and probes)	0.5 µl
--	Control Reaction and additional assays (Multiplex PCR)	--
4.0 µl	**Roche Master** (see Roche manual)	4.0 µl
0.1 µl	**RT Enzyme** (see Roche manual)	0.1 µl
15.0 µl	**Volume of Reaction Mix**	**10.0 µl**

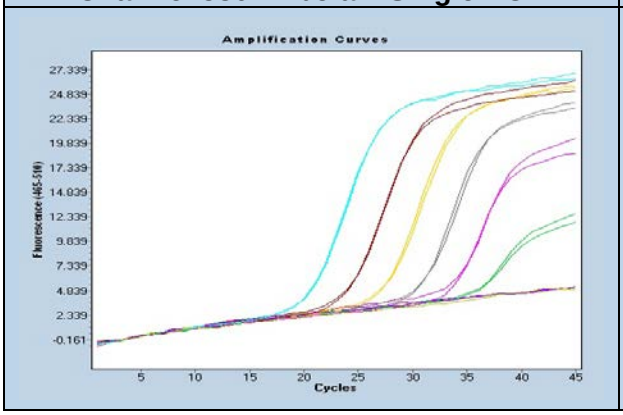
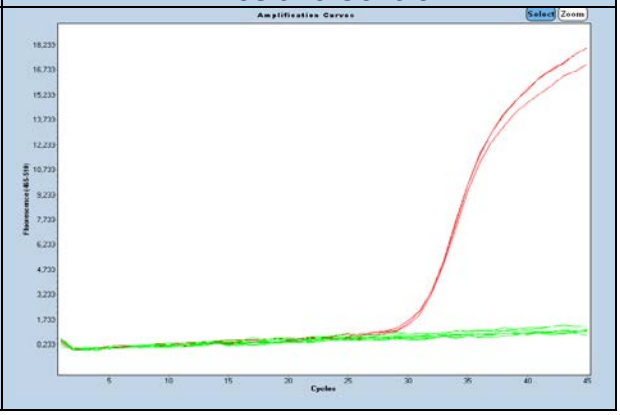
Table 2

Mix gently, spin down and **transfer 15 µl (10 µl)** per well.

Add 5 µ (10 µl) of sample or control to each well for a final reaction volume of 20 µl. Seal plate and centrifuge.

Start run

9. Typical Results (Data from LightCycler® 480 II system)

Channel 530 : Ebola – Single PCR	Positive Control
	

Dilution row 1E6 to 10 copies / reaction

Figure 1

10. Reading the Results

Perform data analysis as described in the operator's manual. For multiplex assays select the color compensation. We recommend using the Second Derivative Maximum method (Automated (F'' max). View results in the FAM channel. The negative control (NTC) must show no signal.

Channel 530 (sample)	Channel 660 Control Reaction	Channel 530 NTC Control	Result
No amplification	Detectable	Negative	Not detectable
Amplification Cp < 39+	Not relevant	Negative	Ebola Positive
No amplification	Not detectable	Not relevant	PCR failure Repeat
Amplification signal	Not relevant	Positive	Contamination Repeat

Note: cobas z 480 Analyzer signal levels are about 50% compared to LightCycler® 480 II results.
+ Recommendation : Define the cut-off 2-4 cycles higher than observed for 10 copies

11. References

Diagnostic reverse-transcription polymerase chain reaction kit for filoviruses based on the strain collections of all European biosafety level 4 laboratories. Panning et al. J Infect Dis. 2007

12. Multiplex PCR Compatibility

This assay can be combined with other assays including an internal control (IC) or a spiked extraction control (for example PhHV or EAV) as depicted below :

Respiratory Multiplex PCR and Instrument Compatibility

Color Compensation 40-0320 is mandatory for Multiplex PCR

500	530	580	610	640	660
	EBOV				MSTN or
	EBOV				PhHV or
	EBOV				EAV

480 II	z 480	LC96	LC2.0	Nano
X	X	X		X
X	X	X		X
X	X	X		X

Table 3

13. Version History

V140404	Release version	2014-04-14
V140808	Positive control is now RNA	2014-08-08
V140909	Editorial changes	2014-09-09
V151001	2015 protocol Multiplex Master, 5/10 µl extract and 60°C acquisition	2015-10-01
V160313	1. Storage of controls, 8.2.2 buffer, 8.2.3 wording	2016-06-10

Note. EU / German Export Restrictions for this product (Dual Use Bioweapon Detection). End-user-certificate may be required. End user will be reported to the National Authorities

Certificate of Analysis (CoA)

Lot n°

Expiry :

Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range	21-23	24-26	27-29	30-32	33-36	36-38	
Measured							
Signal level				40-60			
Measured							
Negatives	10/10						✓

***Note**:* Cp (crossing point) values collected with pDNA (single target PCR). Fluorescence (FL) levels depend on instrument settings and may vary. 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 (ΔCp).

QC Acceptance Date: YYYYMMDD

We, the undersigned, certify that the product designated above has been obtained in accordance with the rules of production and quality control.

Name(s) :

TIB MOLBIOL Syntheselabor GmbH | Eresburgstr. 22-23 | D-12103 Berlin | Germany
Tel. +49 30 78 79 94 55 | FAX +49 78 79 94 99 | dna@tib-molbiol.de | WWW.TIB-MOLBIOL.COM
Geschäftsführer (CEO): Olfert Landt | Register HRB 93163 B | Registergericht Berlin Charlottenburg