TIB MOLBIOL

Instructions For Use

LightMix® Modular RNase P RNA

580

Cat.-No. 58-0911-96 Roche SAP n° 07 683 294 001

Kit with reagents for 96 PCR reactions 20 µl for detection of RNase P RNA [lyophilized]

1. Content, and Storage and Expiry

Storage at Arrival:

1 Vial red cap 96 reactions RNase P RNA (lyophilized)

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.

2. Additional Reagents required

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

3. Introduction

PCR analysis of biological samples may fail due to problems related to the nucleic acid extraction. In particular, false negative results in pathogen testing can be due to the presence of inhibitors, degraded target material or an unsuccessful extraction of nucleic acids. The presence of amplifiable nucleic acids can be verified by running an extraction control PCR reaction.

This product is intended to be used as extraction control for RNA-based PCR assays containing human mRNA. The preferred sample type are for example swabs, containing low amounts of human material; samples extracted for example from blood may contain too much target thus the RNase P assay may outcompete the analytical assay. The detection limit of the analytical assay has to be tested in combination with the control assay. This assay is derived from a published CDC assay, with one primer shifted to be transcript-specific - this kit will different from the CDC assay not work with genomic DNA.

4. Description

A 89 bp long fragment from the human RNaseP mRNA transcript is amplified with specific primers and detected with a R6G labeled hydrolysis probe (580 channel).

5. Specification

These reagents detect 1,000 copies or less transcript.

6. Sample Material and Extraction

Depends on the analytical PCR. 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 580 Channel **Set Quant Factor 10, Max Integration time 1 sec**
LightCycler® 480 Instrument: 523-568
LightCycler® 480 II Instrument: 533-580
cobas z 480 Analyzer (open channel): 540-580

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 RNA with water.

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 red 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 Target RNA

This control assay works with mRNA. Follow the procedure for the analytical PCR

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. The table contains the amounts for a duplex reaction for one analytical parameter (red) and the control:

Duplex PCR - Instruction for use with the Roche LightCycler® Multiplex RNA Virus Master		
for 5 µl extract	**Component**	**10 µl extract**
9.9 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	4.9 µl
0.5 µl	**RNase P Control Reaction** and	0.5 µl
0.5 µl	**Reagent** mix additional assay(s) (Multiplex PCR))	0.5 µl
--	LightCycler® 1.x / 2.0 optional : 3 µl Seek dye (substitute water)	--
4.0 µl	**Roche Master** (see Roche manual)	4.0 µl
0.1 µl	**RT Enzyme** (RNA Master only)	0.1 µl
15.0 µl	**Volume of Reaction Mix** (DNA Master 14.9 µl)	**15.0 µl**

Table 2

Mix gently, spin down and **transfer 15 µl (10 µl)** per well.
Add 5 µl (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)

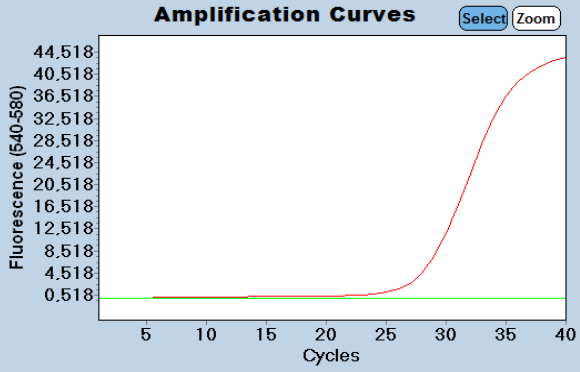
Channel 580 : RNaseP – Single PCR	Data obtained in Multiplex PCR
	

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 660 channel. The negative control (NTC) and negative samples must show a signal.

Channel 580 Control Reaction	Other channel Analytical PCR	Analytical PCR NTC Control	Result
Amplification signal +	Not detectable	Negative	Parameter - Negative
Not relevant	Amplification signal	Negative	Parameter- Positive
No amplification	Not detectable	Negative	**PCR failure Repeat**
Not relevant	Not relevant	Positive	Contamination Repeat

Notes: 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

Real-Time Reverse Transcription–Polymerase Chain Reaction Assay for SARS-associated Coronavirus. Emery et al., Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 10, No. 2 (2004)

12. Multiplex PCR Compatibility

The RNase P extraction control assay can be combined with up to three (LightCycler® 96), four (cobas z 480) or five (LightCycler® 480 system) analytical assays :

Multiplex PCR and Instrument Compatibility

Color Compensation 40-0320 is mandatory for Multiplex PCR

500	530	580	610	640	660	480 II	z 480	LC96	LC2.0	Nano
	Assay 1	RNaseP				X	X	X	X	X
	Assay 1	RNaseP	Assay 2		Assay 3	X	X	X		
	Assay 1	RNaseP	Assay 2	Assay 4	Assay 3	X	X			
Assay 5	Assay 1	RNaseP	Assay 2	Assay 4	Assay 3	X				

Table 3

13. Version History

V150303	Release version	2014-03-01
V150505	Roche SAP number	2015-05-12
V151001	2015 protocol Multiplex Master, 5/10 µl extract and 60°C acquisition	2015-10-01
V160313	8.2.3 wording	2016-07-07

Certificate of Analysis (CoA)

Lot n°
Expiry :

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Dilution	**PSR**	**passed**
Cp range Measured	28-30	
Signal level Measured	40-50	
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:

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

Name(s) :

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