TIB MOLBIOL

LightMix® Modular Hepatitis E Virus (HEV) FAM

Cat.-No. 53-0638-96 Roche SAP n°07225229001

Kit with reagents for 96 PCR reactions 20 µl for detection of HEV genomic RNA [lyophilized]

1. Content and Storage at Arrival:

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

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

2. Storage and Expiry

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

2. Additional Reagents required

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

3. Introduction

HEV is an uncoated plus single-stranded RNA virus of the family *Hepeviridae* causing Hepatitis E in humans. The disease is more severe than Hepatitis A. The virus is transmitted by the fecal-oral route.

4. Description

A 70 bp long fragment from the viral capsid gene is amplified with specific primers and detected with a FAM labeled hydrolysis probe (530 channel).

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 serum or 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 LightCycler® 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 **Set Quant Factor 10** (default setting is 1)

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]	5 min	00:05:00	00:00:15	00:00:30	00:00:02	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	None	**Single**	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.
- **Positive control:** Run a positive control - replace the template RNA with the provided control RNA.

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

One reagent vial with a **yellow** cap contains all primers and probes 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 RNA

Add 160 µl PCR-grade water to the vial with the **black** cap. Mix by pipetting the solution up and down 10 times. **Note:** Opening of this vial may cause contaminations of the work-space (aerosol).

►**Use 5 µl** positive control RNA for a 20 µl PCR reaction (1,000 copies / 5µl).

8.2.3. Preparation of the Reaction Mix

In a cooled reaction tube, prepare the reaction mix for single reactions (left) or one plate (right):

For use with the Roche LightCycler® Multiplex RNA Virus Master		
One reaction	**Component**	**100 reactions**
10.4µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	1040 µl
0.5 µl	**Reagent** mix (parameter specific reagents containing primers and probes)	50 µl
--	Control Reaction and additional assays (Multiplex PCR)	--
4.0 µl	**Roche Master** (see Roche manual)	400 µl
0.1 µl	**RT Enzyme** (see Roche manual)	10 µl
15.0 µl	**Volume of Reaction Mix**	**1.500 µl**

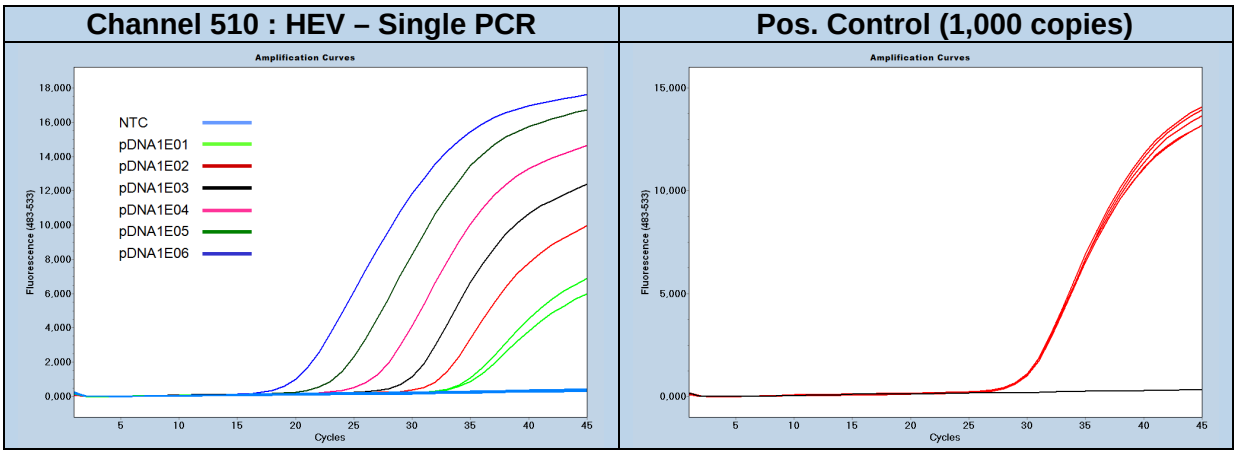
Table 2

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

Add 5 µl of sample or control DNA 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)



Dilution row 10E6 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 510 (sample)	Channel 660 Control Reaction	Channel 510 NTC Control	Result
No amplification	**Detectable**	**Negative**	**Not detectable**
Amplification Cp < 39 +	**Not relevant**	**Negative**	**HEV Positive**
No amplification	**Not detectable**	**Not relevant**	**PCR failure Repeat**
Amplification signal	**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

A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. N. Jothikumar, T.L. Cromeans, B.H. Robertson, X.J. Meng, V.R. Hill. Journal of Virological Methods (2006)

12. Multiplex PCR Compatibility

This HEV assay can be combined with other assays up to 6plex reactions including an internal control (IC), an extraction control or a spiked extraction control (for example PhHV or EAV) as depicted below :

Multiplex PCR and Instrument Compatibility

Color Compensation 40-0320 is mandatory for Multiplex PCR

500	FAM	580	610	640	660
	HEV	control			
	HEV				EAV or PhHV
	HEV				

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

Table 3

13. Version History

V140404	Release version	2014-04-14
V140909	Editorial changes	2014-09-09

Certificate of Analysis (CoA)

Lot n°
Expiry :

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Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range				29-31			
Measured							
Signal level				25-40			
Measured							
Negatives	10/10						✓

***Note**:* Fluorescence (FL) levels depend on instrument settings and may vary. The crossing point (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) :

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