For life science research use only. Not for use in diagnostic procedures. For *in vitro* use only.

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# LightMix® Modular MERS-CoV Orf1a

**FAM**

Cat.-No. 53-0635-96 Roche SAP n°07088833001

Kit with reagents for 96 PCR reactions 20 µl for detection of MERS Corona virus [lyophilized]

## 1. Content and

**1** Vial yellow cap 96 reactions orf1a (lyophilized)
**1** Vial black cap Positive Control (32,000 copies)

## Storage at Arrival:

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 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

Middle East respiratory syndrome coronavirus (MERS-CoV) has been first reported 2012 in Saudi Arabia with currenly (2014-14) more than 200 cases and 89 deaths, most of them in Arabian countries. Positive laboratory results obtained with the upE gene specific test (kit 53-0624-96) should be confirmed with a test for the orf1 gene (this kit).

## 4. Description

A 84 bp long fragment upstream of the Orf1a 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.

## 6. Sample Material and Extraction

Typical clinical samples are nasopharyngeal swabs, tracheal aspirates or bronchoalveolar lavage.
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.

### 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** 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 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** |

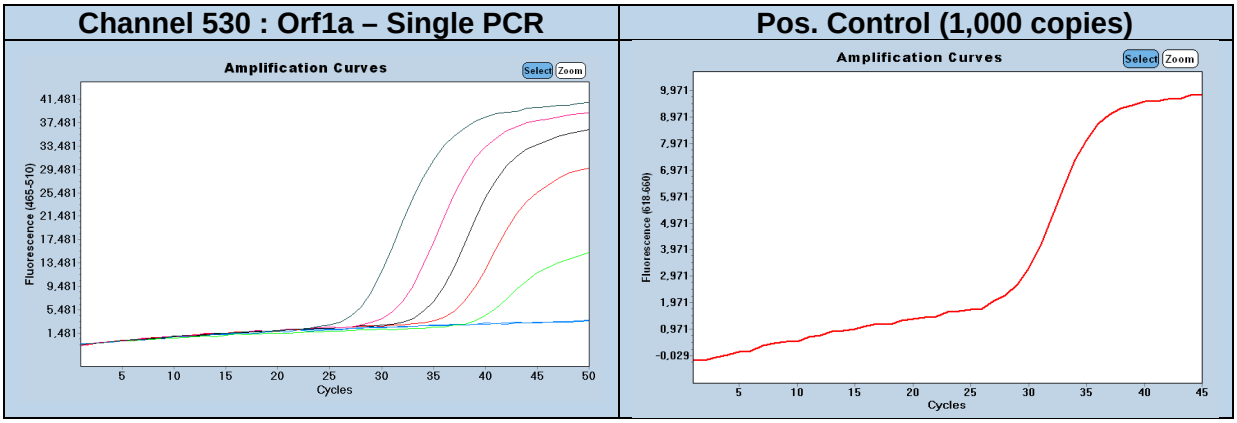
Table 2

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

**Add 5 µl** of sample or control 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** | **MERS-CoV 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


Detection of a novel human coronavirus by real-time RT-PCR. Corman et al., Eurosurvaillance (2012)
http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20285

## 12. Multiplex PCR Compatibility Respiratory Virus Panel

This Orf1a assay can be combined with other assays up to 6plex reactions including an internal control (IC) or a spiked extraction control (for example PhHV) as depicted below :

**Respiratory Multiplex PCR and Instrument Compatibility**

Color Compensation 40-0320 is mandatory for Multiplex PCR

| 500 | FAM | 580 | 610 | 640 | 660 | 480 II | z 480 | LC96 | LC2.0 | Nano |
|---|---|---|---|---|---|---|---|---|---|---|
| | orf1a | | | | MSTN or | X | X | X | | X |
| | orf1a | | | | PhHV | 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 | | | | 28-30 | | | |
| Measured | | | | | | | |
| Signal level | | | | | | | |
| 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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