User Manual

Tetraplex Real-Time PCR AllCovid 2

Principle

This method describes a routine procedure for quantitative detection of DNA of the elements stated below. Namely for Covid (CDC- Primer for N1-gene, N2 and E) and the Pepper Mild Mottle Virus (PMMV), the reverse-transcription real-time PCR. In addition to the primers specific to each individual system, two differently labelled Taqman-probes are used for fluorescence optical detection:

Covid-19 N1	**FAM**
PMMV	**HEX**
COVID 19 N2	**ROX**
COVID 19 E	**CY5**

The number of cycles at which the measured fluorescence signal exceeds a predefined threshold value results in the Ct-value (cycle threshold). Quantification of the samples to be measured is achieved via an external calibration series.

IMPORTANT: The kit may only be used for environmental samples, not for human samples.

Contents and Storage

5 tubes of primer-probe mix, lyophilized, for 5x20 reactions. Shipped at ambient temperature, store at -20°C, do not expose to light.

Reagents to be Supplied by User

Reliance One-Step Multiplex RT-qPCR Supermix (CatNo 12010176 200x) available from BioRad. Good results are also achieved with SOLIScript® 1-step Multiplex Probe Kit from Solis Biodyne (Cat No. 08-55-00250 200x,).

Available upon request: Template-file for Rotorgene 6000 cycler, evaluation table EXCEL

Protocol

1. Add 187 µl water (PCR grade) per tube of supplied master mix, vortex vigorously (store solution at 4°C, do not expose to light, stable for 1 week).

2. Add 133 µl Reliance One-Step Multiplex RT-qPCR Supermix or respective amount of similar product and mix well.
Yields 320 µl ready-to-use mastermix for 20 reactions à 25 µl reaction volume.

3. Mix 15 µl ready-to-use mastermix with 10 µl of the diluted viral RNA solution to be tested in a suitable PCR reaction vessel.
Note: Perform all steps in a RNase-free manner (wear gloves, avoid dust if possible).

4. Set up your Real-Time PCR machine according to the manufacturer in order to be able to measure the used fluorescence dyes.

5. Use the following thermal cycling profile:

1 10 min, 50°C
2 10 min, 95°C
3 5 s, 95°C
4 30 s, 57°C
5 Repeat steps 3 to 4 **40 times in total**

6. Analyze the fluorescence traces according to the manufacturer of your Real-Time PCR machine and determine the Ct-values and the amount of target DNA in each sample by comparing to known standards.

Contact

Kantonales Labor Zürich
rene.koeppel@klzh.ch

Further Information

https://www.microsynth.com/allcovid.html

Microsynth AG
Schützenstrasse 15
CH-9436 Balgach
Telefon +41 71 722 83 33
Fax +41 71 722 87 58
www.microsynth.com

March 2022