# Implementation of SARS-CoV-2 (Coronavirus) detection assays from TIB MOLBIOL on qTOWER³ for research use

## Introduction

December 2019 the novel SARSCoV-2 (formerly named 2019-nCoV) was identified in Wuhan, the capital of China's province Hubei. Based on its rapid spreading with more than 950,000 confirmed cases by the beginning of April 2020 the World Health Organization declared the outbreak a public health emergency of international concern.

**Your Benefits**

- Ideal real time PCR signals on qTOWER³
- Instrumentation for detection of SARS, SARS-CoV-2 and other bat-associated SARS-related viruses
- Instrumentation for detection of SARS-CoV-2 specific sequence (via RdRP gene
- Maximum flexibility on qTOWER³

## Application

Molecular assays to detect SARS-CoV-2 have been developed and are accessible through the homepage of the WHO. Furthermore, several commercial kits based on real-time PCR are available like the Modular Real-time PCR assay from the company TIB MOL BIOL:
- TIB MOLBIOL: **LightMix Modular SARS-CoV (COVID19) E-gene 53-0776-96**
- TIB MOLBIOL: **LightMix Modular SARS-CoV-2 (COVID19) RdRP 53-0777-96**
- TIB MOLBIOL: **LightMix Modular EAV RNA Extraction Control 66-0909-96**

In scope of general corona virus identification the assay specific for the E-Gene (53-0776-96) is in focus. Here a 76 bp long fragment from a conserved region in the E gene is detected with FAM labeled hydrolysis probes (530 channel). This assay will detect SARS and SARS-CoV-2 as well as other bat-associated SARS-related viruses (Sarbecovirus). The first assay will be followed by confirmatory testing with the RdRP gene (53-0777-96) assay. The application of the RdRP gene assay detects the specific SARS-CoV-2 sequence, here a 87 bp long fragment from a conserved region of the RNA-dependent RNA polymerase (RdRP) gene is detected with a SARS-CoV-2 specific FAM labeled hydrolysis probes.

Both assays can be combined with the Lightmix MODULAR EAV RNA Extraction control.

The following results show the compatibility of the indicated TIB MOL BIOL assays in combination with Analytik Jena qTOWER³ for the research use-based detection of SARS-CoV-2 or SARS-CoV-2, SARS and other bat-associated SARS-related viruses (Sarbecovirus).

## Materials and reagents

Both **LightMix Modular SARS-CoV (COVID19) E-gene** (53-0776-96) and **LightMix Modular SARS-CoV-2 (COVID19) RdRP** (53-0777-96) by TIB MOLBIOL were used. For the experiments Control **ivRNA** (nCoV-Mix ivRNA) with different dilutions were used for evaluation in qTOWER³. As indicated in respective manuals LightCycler Multiplex RNA Virus Master (06 754 155 001) by Roche was used additionally. Assays were implemented on the qTOWER³ by Analytik Jena. The instrument's setting can be seen in figure 1.

The setting of qPCRsoft:

| 6 steps | scan | °C | m:s | goto | loops | ΔT(°C) | Δt(s) | ↗(°C/s) |
|---------|------|------|-------|------|-------|--------|-------|---------|
| 1 | | 55,0 | 05:00 | -- | --- | --,- | --- | 8,0 |
| 2 | | 95,0 | 05:00 | -- | --- | --,- | --- | 8,0 |
| 3 | | 95,0 | 00:05 | -- | --- | --,- | --- | 8,0 |
| 45x 4 | ◆ | 60,0 | 00:15 | -- | --- | --,- | --- | 6,0 |
| 5 | | 72,0 | 00:15 | 3 | 44 | --,- | --- | 6,0 |
| 6 | | 40,0 | 00:30 | -- | --- | --,- | --- | 6,0 |
| 7 | | | | | | | | |
| 8 | | | | | | | | |
| 9 | | | | | | | | |
| 10 | | | | | | | | |

| Pos. | Channel | Excitation | Detection | Dye | Gain | Measureme | Pass. Ref. |
|------|---------|------------|-----------|-----|------|-----------|------------|
| 1 | Blue | 470nm | 520nm | FAM | 5 | ◆ | |
| 5 | Red | 630nm | 670nm | Cy5 | 5 | ◆ | |

Figure 1: Overview in the settings of qTower³ by qPCRsoft.

## Results

### TIB MOLBIOL: LightMix Modular SARS-CoV (COVID19) E-gene and EAV

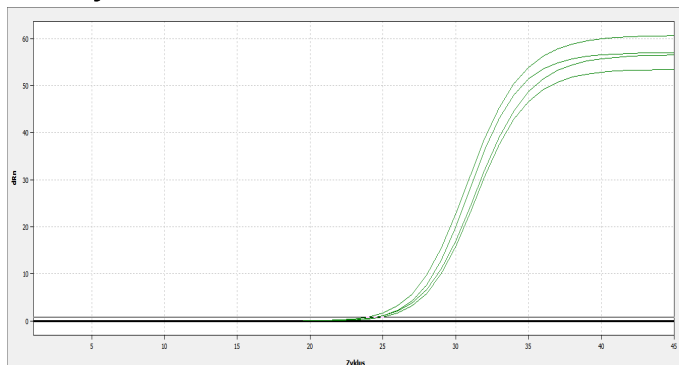| E-Gene: FAM channel | Table 1: Detection of Sarbeco control in different dilutions in FAM channel |
|---------------------|------------------------------------------------------------------------------|
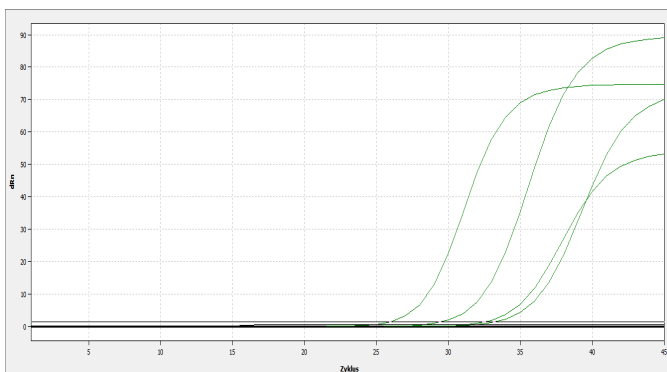


Amplification of conserved E-Gene: detection of SARS and SARS-CoV-2 as well as other bat-associated SARS-related viruses (Sarbecovirus).

| Sample name | Ct value FAM |
|-------------|--------------|
| sPCR Sarbeco 1:100 | 33.35 |
| sPCR Sarbeco 1:100 | 32.54 |
| sPCR Sarbeco 1:10 | 29.54 |
| sPCR Sarbeco | 26.01 |

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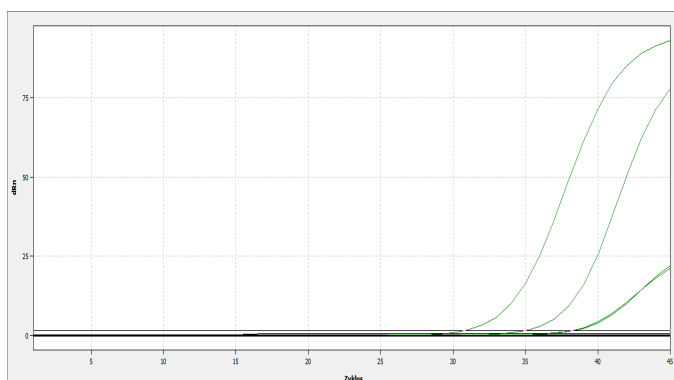
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## EAV: Cy5 channel



Amplification of extraction control in Cy5 channel.

Table 2 : Detection of EAV in Cy5 channel

| Sample name | Ct value Cy5 |
|---|---|
| sPCR Sarbeco 1:100 | 24.89 |
| sPCR Sarbeco 1:100 | 24.52 |
| sPCR Sarbeco 1:10 | 24.48 |
| sPCR Sarbeco | 23.84 |

## TIB MOLBIOL: LightMix Modular SARS-CoV-2 (COVID19) RdRP and EAV
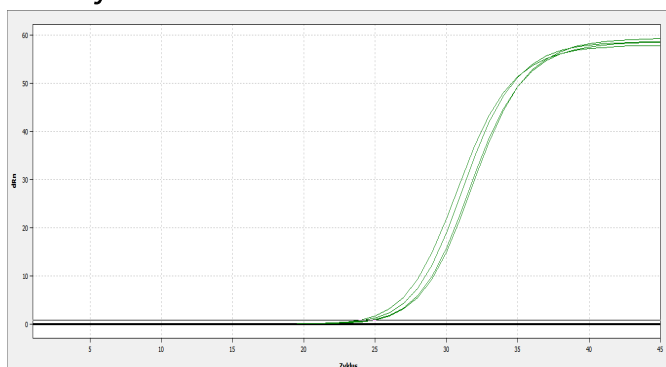
### RdRP-Gene: FAM channel



Amplification of RdRP-Gene: specific detection of SARS-Cov2.

Table 1: Detection of Sarbeco control in different dilutions in FAM channel

| Sample name | Ct value FAM |
|---|---|
| sPCR Sarbeco 1:100 | 38.24 |
| sPCR Sarbeco 1:100 | 38.11 |
| sPCR Sarbeco 1:10 | 35.03 |
| sPCR Sarbeco | 30.67 |

### EAV: Cy5 channel



Amplification of extraction control in Cy5 channel.

Table 2 : Detection of EAV in Cy5 channel

| Sample name | Ct value Cy5 |
|---|---|
| sPCR Sarbeco 1:100 | 24.90 |
| sPCR Sarbeco 1:100 | 24.78 |
| sPCR Sarbeco 1:10 | 24.37 |
| sPCR Sarbeco | 23.88 |

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## Conclusion:

Both Genes, E-Gene and the SARS-CoV2-specific RdRP-Gene can be detected by the usage of the indicated assays instrumented on the qTOWER³. Please note that qTOWER³ is intended for research use only.

## Literature:

Corman VM et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR