SARS-CoV-2-E-DETECT
Datasheet
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For research use only

Intended Use
SARS-CoV-2-E-DETECT Kit is used for the qualitative detection of Betacoronavirus through Reverse Transcription and Real-Time Polymerase Chain Reaction (real-time RT-PCR) of the E-gene in respiratory specimens.

Product-code	CoV-2-E-D250
Pack Size	250 reactions
Reaction Volume	20 µL

Kit Contents

Reagents	250 reactions/Kit
5 x Reaction-Mix*	1.000 µL
40 x Enzyme Mix* for 1-step real-time RT-PCR	125 µL
Primer-Probe Mix specific for E-gene SARS-CoV-2 and for Internal Control (human RNaseP-Gene)	1250 µL
Recombinant Positive Control (1.0E+04 copies/rxn)	125 µL
H_2O	5 mL

*Solis BioDyne

Description
SARS-CoV-2-E DETECT contains a premixed ready-to-use Reaction-Mix, an Enzyme Mix, the Multiplex-Primer-Probe Mix of the E-gene and of the internal extraction control (human RNaseP-Gene) for performing one-step Reverse Transcription real-time PCR.
The probe specific for the E-gene is labeled with FAM, the probe specific for the Internal Control is labeled with Cy5.
The internal control detects the human RNaseP-Gene and serves as a swab-extraction control, i.e. correct amplification indicates correct sampling and nucleic acid extraction.
The positive control determines correct amplification of the E-gene.
Caution: To avoid cross contamination to other samples care should be taken when adding the positive control.

Storage
Routine storage: -20°C. Shipping and temporary storage for up to 7 days at room temperature.

Additional required materials and devices, not provided
Real Time PCR Instrument (capable for the detection of FAM and Cy5)
Pipettes (1-20µL, 10-200µL, 100-1,000µL), Pipettes tips with aerosol barrier (RNase, DNase free)
Powder-free gloves disposable
Vortex mixer
Sterile microtubes
Bench microcentrifuge
RNA Isolation Kit

RNA-extraction
A good quality of the extracted RNA is very important for the performance of the real-time RT-PCR. The use of carrier RNA is crucial for extraction efficiency and stability of the extracted RNA.
Different brands of RNA extraction kits are available. Please comply with manufacturer's instructions.
If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of PCR.

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Real Time RT-PCR Protocol

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

1. Mix 4 µL Reaction-Mix, 5 µL Primer-Probe-Mix and 0,5 µL Enzyme-Mix to prepare RT-PCR Master Mix as described in the following table.

Adjust the volume of the RT-PCR Master Mix for all samples plus an extra sample to compensate for small pipetting errors. Volume Total RT-PCR Master Mix

= n samples + 1 positive control + 1 NTC (non template control) + 1 additional rxn

Component	1 Reaction
5 x Reaction Mix*	4 µL
40 x Enzyme Mix*	0,5 µL
Primer-Probe Mix	5 µL
H_2O ad 15µL	5,5 µL
Total	15 µL

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2. Place 15µL of RT-PCR Master mixture into each PCR tube.
3. Add 5 µL of each sample RNA into the corresponding PCR tube for amplification.
4. Place 5µL of Positive Control and 5 µL H_2O as Negative Control (NTC) into the corresponding PCR tube.

Caution: To avoid cross contamination to other samples care should be taken when adding the positive control.

5. Mix, spin and transfer PCR tube for testing into the real time thermal cycler.
6. Perform the following protocol in the instrument:

Step	Temperature	Time	Cycles
Reverse transcription	55°C	15 min	1 cycle
Initial Denaturation	95°C	10 min	1 cycle
Denaturation	95°C	15 sec	45 cycles
Extension	58°C	1 min	

Fluorescence (FAM, Cy5) is measured at the extension step.

Please ensure that the instrument used has been installed, calibrated, checked and maintained according to the manufacturer's instructions.

Data Analysis and Interpretation

Negative control, positive control and internal control must be detected correctly, otherwise the sample results are invalid.

Detection Channel	Template	Results		
FAM	Positive Control	positive	positive	positive
FAM	NTC	negative	negative	negative
FAM	Unknown Sample	negative	positive	positive
Cy5	IC[a]	positive	positive	positive/negative
Result Interpretation		**negative**	**positive**	**positive**

IC[a] = Detection of the IC may be decreased or absent in samples with higher amount of SARS-CoV-2 RNA due to competitive amplification.

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