Coronavirus (SARS-CoV-2)

Real-time qPCR Detection kit

(100 rxns)

Zellbio GmbH (Germany)

CAT No. ZX-22100-100

www.zellx.de

ZellX PCR Primer & Probe sets are designed based on publicly available 2019-nCoV real-time PCR protocols for Emergency Use Authorization (EUA) by Center for Disease Control and Prevention (CDC) in the USA. More information at https://www.fda.gov/media/134922/download.

Sample Types Validated for:

Respiratory specimens (sputum; nasopharyngeal, oropharyngeal aspirates, washes or swabs; tracheal aspirates)

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

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Please read this insert completely prior to using the product.

Background

A novel human coronavirus, currently named 2019-nCoV, causes ongoing pneumonia outbreak which started in Wuhan City in China. The 2019-nCoV is genetically different from other common human coronaviruses such as 229E, NL63, OC43 and HKU1 that cause seasonal acute respiratory illnesses. It is also genetically distinct from the two newer human coronaviruses, MERS-CoV and SARS-CoV.

The ZellX® SARS-CoV-2 RT-qPCR Kit is designed for quantitative *in vitro* detection of SARS-CoV-2 using real time RT-PCR in respiratory specimens (sputum; nasopharyngeal, oropharyngeal aspirates, washes or swabs; tracheal aspirates).

The ZellX® RT-qPCR Kit allows efficient cDNA synthesis and Real-Time PCR in a single tube. The qPCR Master Mix provided in the kit contains all the reagent (except PCR primers, probe and template) needed for running PCR reactions. To prevent the degradation of viral RNA by RNases, our kit comes with a vial of Ribonuclease (RNAse) inhibitor.

Our kit also contains one set of primers and fluorescent probe designed according to target region of the virus nucleocapsid (N) gene and to detect the human RNase P used as a control to assess specimen quality. The probes have readout in different channels. Coronavirus SARS-CoV-2 RNA targets are amplified and detected in the FAM channel, while human RNase P RNA targets are amplified and detected in the HEX, VIC or JOE channel (depending on the equipment).

The assay does not include a positive control (PC). A PC is needed to demonstrate that the PCR amplification is working efficiently with the supplied components. To confirm absence of contamination, a non-template (negative) control reaction should be included every time the kit is used.

Materials supplied in the Kit

Component	*Quantity*
qPCR Master Mix (4X) lyophilized	1 vial
Ribonuclease inhibitor	1 vial
Primer/ Probe set	1 vial
Reconstitution Solution	1 vial

Storage instruction

All reagents should be stored at -20°C upon receipt. Avoid repeated freezing and thawing.

primer/probe Mix must be kept in the dark. Mix gently, aliquot and store at -20°C. **Avoid repeated freeze and thaw cycles.**

Materials required but not supplied

Real-time PCR detection system equipped for FAM (465-510) and VIC / HEX / JOE (533-580) detection

Precision pipettes and disposable filter pipette tips (RNase & DNase free)

Appropriate PSA & workspaces for working with potentially infectious samples

Nuclease-free tubes / strips / plates corresponding to the PCR device

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- The instruction must be strictly followed. The PCR machine must be turned on and programmed in advance to avoid delays after setting up the reactions.
- Pipette tips should not be used more than once to prevent cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Reagent preparation

qPCR Master Mix (4X): Add 500 µL of the Reconstitution Solution to the qPCR Master Mix vial, mix well by pipetting until it get completely dissolved, and keep it at -20°C.

Sample preparation

The results of RT-PCR assays are strongly depends on the quality and amount of RNA. Unsuitable collection, storage and/or transport of specimens may give false negative results. We strongly recommended to qualify and validate RNA quality prior performing assay. **For isolating Viral RNA efficiently use our Viral RNA Extraction Kit Cat. No. ZX-22101-500.**

All samples must be used immediately after preparation or stored at ≤ -70 for later analysis. Avoid repeated freeze and thaw cycles as it decreases the RNA quality and may interfere with the results.

Assay Procedure

For 20 µl reaction

- Thaw all kit components on ice and mix them well. Collect liquid at the bottom of the tube with a quick spin.
- Set up the following reaction mixture.

Component	*Quantity*
qPCR Master Mix	5 µL
Ribonuclease inhibitor	0.5 µL
Primer/ Probe set	5 µL
Nuclease Free water	4.5 µL

Multiply all numbers according to experimental requirements

- Add 15 µL of reaction mix into the number of wells required for your testing. (include 1 well for the NTC and 1 well for the PC)
- Add 5 µL of RNA extracted from each sample, NTC and PC in different wells and close them with the provided caps.
- Program the appropriate PCR cycling protocol on your real-time PCR instrument

Step	*Number of Cycles*	*Temperature*	*Duration*
Reverse transcription	1	42°C	30 min
Initial activation	1	95°C	3 min
Amplification*	4 5	95°C	15 sec
		55°C	30 sec

**Acquisition must be performed at the end of this stage*

- Select the fluorescent channel (FAM/ HEX) of instrument for testing.
- SARS-CoV-2 = FAM (465-510)
- RNase P = VIC / HEX / JOE (533-580).

Interpretation of results

Follow instrument software instructions to generate cycle threshold (Ct) values from the acquired data.

Controls:

- **Non-Template Control (NTC)** should be negative, and must not give Ct value of less than 40 in FAM channel. If NTC reaction is positive, sample contamination has been occurred.
- **Positive Control (PC)** should be positive, and must give a Ct of less than 40 in FAM channel. If these results are not obtained, repeat the assay implementing corrective actions for failed reactions.
- **RNAse P Extraction Control** should be positive and exhibited an expected Ct value for HEX, VIC or JOE channel.
- If all N gene-based reactions and RNase P reaction are negative for the sample, the result should be considered invalid. But if the N1 reactions are positive, even in the absence of a positive RNAse P, the result should be considered valid.

Samples:

When all controls exhibit the expected performance, then the samples could be positive, negative or suspicious

- **Positive:** If Ct ≤ 40 in FAM channels.
- **Negative:** If there is no Ct value in the FAM channel.
- **Suspicious samples:**

➢ If there is no Ct value in the FAM channels, it is recommended to re-extract RNA. If the result is the same, the sample can be reported as negative.

➢ Samples with Ct value greater than 38, it is recommended to re-extract RNA for RT-PCR. If the result is still less than 40, the sample can be reported as positive, otherwise it is negative.

Material Safety Data (MSDS)

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and the EU Directive 67/548/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).

This product is not hazardous, toxic, or IATA-restricted. This product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.