



Cov2Quant™ SARS-Cov-2 Quantification kit



Cov2Quant™ SARS-Cov-2 Quantification kit
CQ-100 100 tests/kit
STORE AT -20 °C or below
Shelf life is 12 months after manufacturing

One-step RT-QPCR kit



*For ABI7500/7500 Fast/Roche LightCycler® 96
CFX96/CFX384/Mic qPCR Cycler, 4-Channel*



INTENDED USE

Cov2Quant™ SARS-Cov-2 Quantification kit is a One-step reverse transcription real-time PCR (RT-QPCR) kit designed to detect the novel corona virus, SARS-Cov-2, quantitatively by using reverse transcription reaction and quantitative real-time polymerase chain reaction.

KIT COMPONENT

Cov2Quant™ SARS-Cov-2 Quantification kit		100 tests/kit
Yellow	qPCR 1-Step MIX	1,000 µl
Purple	RTase	100 µl
Green	Probe Mixture	410 µl
Blue	Negative Control	100 µl
Red	Positive Control	100 µl

PREREQUISITE

- Applied Biosystems 7500/7500 Fast, Roche LightCycler® 96, CFX96/CFX384 (Bio-Rad) or Mic qPCR Cycler, 4-Channel real-time PCR instrument
- Vortex mixer
- Centrifuge with rotor for microtiter plates
- Pipettes and pipette tips with aerosol barrier
- Disposable powder-free gloves

PRECAUTION AND WARNING

- The kit is designed for research use only (RUO) or in vitro diagnostics (IVD)
- Performing tests has to be done by well-trained and qualified personnel
- Dispose unused reagents and waste according to local regulations
- Do not modify the sample/reagent volume
- Do not mix reagent from different kits or different batches of this kit
- Avoid unnecessary freezing and thawing of the reagents



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PROTOCOL

A) Sample

This kit is designed to detect Sars-Cov-2 viral RNA from total RNA extracted from human respiratory specimen such as naso/pharyngeal swab, sputum, alveolar lavage, using different sampling and RNA purification protocols. Preferred validated IVD kit for sampling: Virus Transport medium with flocked swab – cat no: ATM-1 (Avidin).

B) RNA Extraction

Commercial RNA purification kit, such as AviRNA Viral RNA Extraction Kit (AVE-100, Avidin) is recommended.

C) Reagent Preparation

Before use all components need to be thawed, gently mixed and centrifuged briefly to collect reagent solution at the bottom.

1. Mix 10 µl qPCR 1-Step MIX, 1 µl RTase and 4 µl of Probe Mixture to prepare master mix per each reaction. Prepare app. 10% more volume of master mix for all the reactions to prevent pipetting error
2. Pipette 15 µl master mix into PCR tube or 96-well PCR plate
3. Add 5 µl of extracted RNA sample into PCR tube or 96-well PCR plate, then mix all components by pipetting. Proceed the same step with Negative Control and Positive Control
4. Close the tube with the cap or seal the 96-well PCR plate
5. Centrifuge the tubes or 96-well PCR plate to collect reagent mix at the bottom
6. Transfer the tubes or 96-well PCR plate into the real-time PCR instrument

D) Real-time PCR Setting

This kit is validated on ABI7500/ Roche LightCycler® 96/ Mic qPCR Cyler, 4-Channel real-time PCR instruments.

1. Set on the dedicated real-time PCR software for thermal cycling parameters according to the manual.
2. Set the following PCR program and fluorescence parameters, then click the start „RUN” button.



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Real-time PCR Profile

	Cycle	Temp.	Time
Section 1	1 cycle	50 °C	10 min
	1 cycle	95 °C	2 min
Section 2	45 cycles	95 °C	10 sec
		60 °C*	30 sec

* Select „Collect Data”

FLUORESCENCE SETTING

Target	Fluorophore
RdRp gene	FAM
N gene	JOE/HEX
IC	Cy5
Reference dye	ROX

E) Analysis Setting

The values of fluorescence emitted by the specific probes and by the specific internal control probe during amplification reactions should be analyzed by the instrument software.

F) Result Interpretation

RdRp	N	IC	Assay Result
<41	<41	<35	COVID-19 Positive
<42	U.D	<35	Repeat the test : COVID-19 positive if RdRp<42
U.D	<41	<35	Repeat the test : COVID-19 positive if N<42
U.D	U.D	<35	COVID-19 Negative
U.D	U.D	U.D	Invalid (re-test)

* U.D, Undetermined



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QUALITY CONTROL

Positive Control and Negative Control Ct range should be the following:

FAM	JOE/HEX	CY5	Assay Result
<33	<33	<33	Positive Control
U.D	U.D	U.D	Negative Control

PERFORMANCE

Criteria	Result
Analytical Specificity	12 viral RNA/DNA samples were tested on the Cov2Quant™ SARS-Cov-2 Quantification kit to evaluate possible cross-reactivity. 12 viral RNA/DNA samples with no relation to the detection target of the kit were NEGATIVE - Cross reactivity: 100% Specificity
Analytical Sensitivity	Serial dilution (20,000; 2,000; 200; 20; 2 copies/test) of Sars-Cov-2 RNA (2 batches, 12 repeat) were tested. Analytical Sensitivity: 1) RdRp gene 20 copies/test; 2) N gene 20 copies/test
Repeatability	Repeatability was confirmed with identical standard samples at different time points with different Lot and testers. Criteria of repeatability was CV < 10% of Ct Value
Freeze/Thaw Safety	Freeze/Thaw Safety was confirmed by 10 times of Freeze/Thaw repeat test. Criteria of repeatability was CV < 10% of Ct Value

MANUFACTURER



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DISTRIBUTOR



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