MANUFACTURED BY EURx

roboklon

SARS-CoV-2
RT-qPCR Detection Kit

SARS-CoV-2 RT-qPCR Detection Kit

Component	**Cat. No.** E0430-01 100 reactions, 25 µl each, 2.5 ml [1x] final volume	**Cat. No.** E0430-02 500 reactions, 25 µl each, 12.5 ml [1x] final volume
CoV Buffer Mix (2x), brown test tube	5 x 250 µl	5 x 1,250 µl
CoV Enzyme Mix, orange test tube cap	100 µl	5 x 100 µl
Positive Control *, black test tube cap	20 µl	100 µl
RNase free water, colourless test tube cap	3 x 500 µl	5 x 500 µl

* Positive Control should be stored separately, away from other kit components.

SARS-CoV-2 RT-qPCR Detection Kit plus ROX Solution

Component	**Cat. No.** E0431-01 100 reactions, 25 µl each, 2.5 ml [1x] final volume	**Cat. No.** E0431-02 200 reactions, 25 µl each, 5 ml [1x] final volume
CoV Buffer Mix (2x), brown test tube	5 x 250 µl	5 x 1,250 µl
CoV Enzyme Mix, orange test tube cap	100 µl	5 x 100 µl
ROX Solution, 25 µM	15 µl	60 µl
Positive Control *, black test tube cap	20 µl	100 µl
RNase free water, colourless test tube cap	3 x 500 µl	5 x 500 µl

* Positive Control should be stored separately, away from other kit components.

Storage:
Store at -20˚C in the dark.

SARS-CoV-2 qRT-PCR Detection Kit is a dual-target plus internal positive control system. The kit is designed to detect SARS-CoV-2 coronavirus in samples from patients with symptoms of COVID-19 infection.

The purified genetic material of the virus from throat, nasopharyngeal, saliva swabs etc. is amplified by realtime RT-PCR and detected using SARS-CoV-2-specific, fluorescence labelled probes.

Virus identification is based on highly conserved regions in the ORF1ab and NP genes characteristic for SARS-CoV-2. In addition, the kit includes primers specific for the human ACTB gene (gene encoding beta-actin), which makes it possible to verify the correctness of the collected swab and the quality of RNA purification.

The test meets the requirements of the WHO recommendation "Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases" dated March 2, 2020. The kit shows no cross-reactions against other microorganisms causing respiratory diseases.

Description:

SARS-CoV-2 qRT-PCR Detection Kit is compatible with most commercially available real-time PCR cyclers.

The Probe Enzyme Mix contains unique highly sensitive reverse transcriptase, hot start DNA Polymerase, and RNase inhibitor.

Reverse transcriptase works at an elevated temperature of 50˚C without loss of specificity and sensitivity.

Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either total RNA or mRNA.

There are two variants of the kit: without ROX and with ROX Solution provided separately. The use of ROX passive reference dye is necessary for all real-time PCR cyclers from Applied Biosystems and optional for cyclers from Stratagene. ROX compensates for variations of fluorescent signal between wells due to slight differences in reaction volume and fluorescence fluctuations. ROX is not involved in PCR reaction and does not interfere with real-time PCR on any instrument. Refer to the table below to determine the recommended amount of ROX (25 µM) required for a specific PCR cycler.

ROBOKLON GMBH | ROBERT-RÖSSLE-STR.10 B55 | D-13125 BERLIN | GERMANY
FAX 030 / 310 19 19 7 | PHONE 030 / 318 09 37 2 | WWW.ROBOKLON.DE
MANUFACTURED BY EURx Sp. z o.o. POLAND | WWW.EURX.COM.PL | MADE IN THE EU

MANUFACTURED BY EURx

roboklon

SARS-CoV-2 RT-qPCR Detection Kit

REAL TIME PCR PROTOCOL

qPCR- Protocol

Recommended amounts of ROX for a specific real-time PCR cycler

Instrument	Amount of ROX per 25 µl reaction	Final ROX concentration
Applied Biosystems: 7300, 7900HT, StepOne, StepOnePlus, ABI PRISM 7000 and 7700	0.5 µl	500 nM
Applied Biosystems: 7500, ViiA 7 Stratagene: Mx3000P, Mx3005P, Mx4000	0.5 µl 10 x diluted (in water)	50 nM
PCR machines from other manufacturers: Bio-Rad, Roche, Corbett, Eppendorf, Cepheid, etc.	Not required	-

Preparation of PCR Reaction:

Component	Patient's RNA sample	Negative Control	Positive Control
CoV Buffer Mix (2x)	12.5 µl	12.5 µl	12.5 µl
CoV Enzyme Mix	1 µl	1 µl	1 µl
Positive Control	-	-	5 µl
Purified RNA from patient sample	5 µl	-	-
Water, nuclease free	6.5 µl	11.5 µl	6.5 µl
Total volume	**25 µl**	**25 µl**	**25 µl**

Thermal Cycling Conditions:

Step	Tempera-ture	Time	Number of Cycles
Reverse Transcription	50˚C	15 min	1
Initial Denaturation	95˚C	2 min	1
Denaturation, Annealing, Extension, Data acquisition	95˚C 60˚C	10 s 40 s	40-45

Notes:

Preparation of the sample taken from the patient.
RNA from a swab, saliva or other secretion should be isolated using kits dedicated to viral RNA purification. Follow the instructions recommended by the kit manufacturer.

Preparation of PCR reaction:

1. Determine the number of adjustable reactions. Reactions can be prepared at room temperature. Enzyme Mix should be kept "on ice".
2. Prepare one reaction Master Mix, i.e. mix the appropriate number of reaction ingredients according to table except for RNA from the patient: mix appropriate amounts of CoV Buffer Mix (2x), CoV Enzyme Mix and water in the tube. Before taking CoV Buffer Mix (2x), mix the buffer by pipetting several times.
3. The components of the Master Mix should be mixed by pipetting several times. Don't "vortex" the mixture.
4. Distribute the Master Mix into strip tubes or onto a plate by transferring 20 µl of the solution.
5. Prepare the reactions in the following sequence:
    - 5a. Negative control: add 5 µl of water and close the tube to avoid contamination by the patient's RNA.
    - 5b. Patient RNA samples: add 5 µl of purified RNA and close the tubes.
    - 5c. Positive control: add 5 µl of Positive Control.
6. One positive and one negative control should be set in addition to the patient samples to be tested for each test cycle.
7. Put the prepared reactions into the thermal cycler and program it as follows:

Fluorescence is measured at the stage of connection of starters/extension in three channels:

- FAM for ORF1ab gene,
- JOE for ACTB gene, and
- ROX for NP gene.

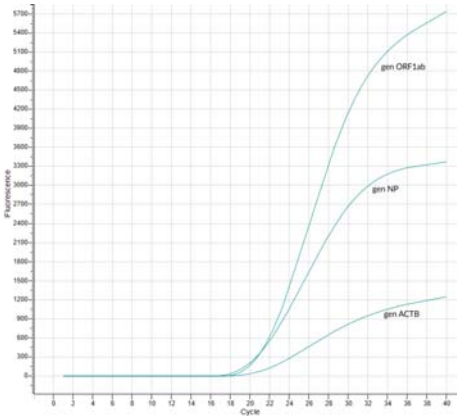
ROBOKLON GMBH | ROBERT-RÖSSLE-STR.10 B55 | D-13125 BERLIN | GERMANY
FAX 030 / 310 19 19 7 | PHONE 030 / 318 09 37 2 | WWW.ROBOKLON.DE
MANUFACTURED BY EURx Sp. z o.o. POLAND | WWW.EURX.COM.PL | MADE IN THE EU

SARS-CoV-2 RT-qPCR Detection Kit

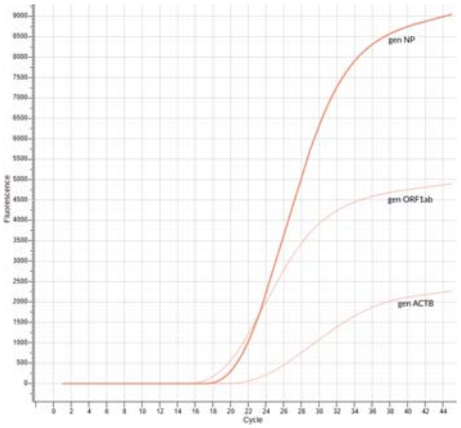
EVALUATION

Example Test Results:

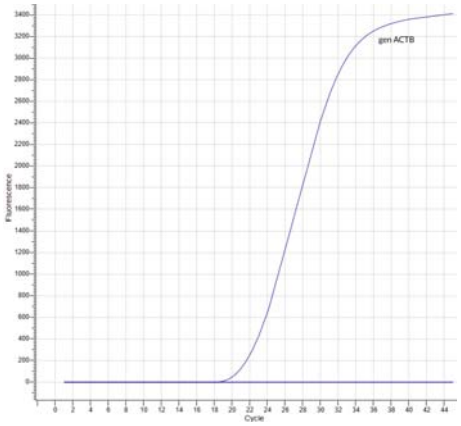
Positive Control:



Positive for SARS-CoV-2:



Negative for SARS-CoV-2:



Evaluation:

Sample Type	FAM channel, ORF1ab (CoV)	JOE channel, ACTB (human)	ROX channel, NP (CoV)	Result
Negative control	-	-	-	correct
Positive control	+	+	+	correct
1	-	+	-	negative for SARS-CoV-2
2	+	+	+	positive for SARS-CoV-2
3	+	+	-	positive for SARS-CoV-2
4	-	+	+	positive for SARS-CoV-2
5	-	-	-	incorrect, repeat the test
6	+	-	+	incorrect, repeat the test

Note:

If you receive an incorrect or a doubtful result, it is recommended to repeat the test.

All tests should be performed by qualified personnel and assessed in the context of clinical symptoms and medical history.

During the test, correct collection of the swab and maintaining high quality of purified RNA is very important.

ROBOKLON GMBH | ROBERT-RÖSSLE-STR.10 B55 | D-13125 BERLIN | GERMANY
FAX 030 / 310 19 19 7 | PHONE 030 / 318 09 37 2 | WWW.ROBOKLON.DE
MANUFACTURED BY EURx Sp. z o.o. POLAND | WWW.EURX.COM.PL | MADE IN THE EU