



EliGene[®] COVID19 UKV/SAV RT

REF

90083-RT (for 100 samples)**90083-RT-500 (for 500 samples)**

Kit components:

90083-RT (for 100 samples):

5 x 300 µl **CoV USV Mix**2 x 55 µl **Enzyme Mix**2 x 260 µl **IC RNA**1 x 150 µl **PC CoV USV**1 x **Instruction for Use**

90083-RT-500 (for 500 samples):

5 x 1450 µl **CoV USV Mix**2 x 280 µl **Enzyme Mix**2 x 1300 µl **IC RNA**1 x 150 µl **PC CoV USV**1 x **Instruction for Use**

Storage and shelf life:

All components of the kit must be transported and stored at -20 °C. Kit and remaining MasterMixes must be stored at -20 °C in a dark.

Intended use

EliGene[®] COVID19 UKV/SAV RT Kit is intended for qualitative RNA detection of SARS-CoV-2 virus, in parallel with the genotyping of the mutations D80A and P681H present in spike protein characteristic for the variant B.1.1.7 (20I/501Y.V1) also called UK variant and B.1.351 (20H/501Y.V2) known as South African variant, respectively.

Principle of the method

This diagnostic kit is based on reverse transcription of viral RNA of SARS-CoV-2 and subsequent one-step qPCR analysis. SARS-CoV-2 detection is carried out by amplifying two independent loci targeting RdRp gene and E gene (FAM channel). A uniquely designed internal control, which is used for the monitoring of the correct course of the sample processing (including RNA isolation, reverse transcription, and qPCR) is recorded in the HEX channel. Specific genotyping probes targeting mutations D80A and P681H are recorded in Cy5 and TexasRed channel, respectively, in parallel with the SARS-CoV-2 detection. Increased sensitivity and specificity of this kit is based on the amplification of multiple independent targets for SARS-CoV-2 virus in a single qPCR reaction.

Introduction

In late December 2019, an outbreak of an unknown disease called “pneumonia of unknown cause” occurred in Wuhan, Hubei Province, China. The causative virus has been named as severe acute respiratory syndrome coronavirus 2 (**SARS-CoV-2**), and the relevant infection disease has been named as coronavirus disease 2019 (COVID-19). Coronaviruses were discovered in the 1960s, and they were classified under the family *Coronaviridae* that is the largest family within the order *Nidovirales*. SARS-CoV-2 is a spherical positive single-stranded RNA virus that is characterized by spike proteins projecting from the virion surface. It is an enveloped virus (envelope is a lipid bilayer derived from the host cell membrane) with the viral structure formed primarily of structural proteins such as spike (S), membrane (M), envelope (E), nucleocapsid (N), and hemagglutinin-esterase (HE). For replication and transcription, multi-protein replicase-transcriptase complex is used. This complex contains conserved RdRp (RNA-dependent RNA polymerase) as main replicase-transcriptase protein for the synthesis of negative-sense subgenomic RNA strands from viral RNA and transcription of negative-sense subgenomic RNA molecules from corresponding positive-sense mRNAs. The RNA genome of coronaviruses is the second largest of all RNA viruses; SARS-CoV-2 has 29,9 kilobases in size.



In December 2020, UK authorities were alerted to the emergence of a SARS-CoV-2 variant of genetic lineage B.1.1.7. The spike protein of the B.1.1.7 lineage harbors 8 mutations: one mutation (N501Y) within the RBD, three mutations (Δ H69/V70, Δ Y144 and A570D) within S1, and four mutations (P681H, T716I, S982A, and D1118H) within S2. The B.1.1.7 variant was isolated in late September and accounted for over 60% of cases in the United Kingdom in early December. According to results of *in silico* analyses of sequenced different genetic lineages of SARS-CoV-2, the occurrence of mutation P681H is specific for genetic lineage B.1.1.7.

In October 2020, lineage B.1.351 (20H/501Y.V2) was identified in South Africa where it quickly prevailed. The B.1.351 variant carries 8 mutations in spike protein: Δ L242/A243/L244, D80A, A701V, D215G, E484K, D614G, K417N, N501Y. The E484K mutation was associated with the reduced protection against infection with B.1.351 variant after the vaccination. *In silico* analysis proved that D80A substitution is unique for the B.1.351 genetic lineage.

Primary sample collection, handling and storage

Clinical material:	Recommended RNA isolation procedure:
nasopharyngeal swabs,	Manual: EliGene Viral RNA/DNA FAST Isolation kit (15 min protocol)
swabs, saliva, sputum,	chemagic Viral DNA/RNA Kit (chemagen - PerkinElmer)
urine	QIAamp Virus Spin Kit or kits recommended by Qiagen
	Vacuum/centrifugation: EliGene Viral RNA/DNA FAST 96 Vacuum Isolation Kit (<40 min/96 samples protocol)
serum, plasma	chemagic Viral DNA/RNA Kit (chemagen - PerkinElmer)
	QIAamp Virus Spin Kit or kits recommended by Qiagen
Automatic isolation:	ZEPHYRUS Magneto (ELISABETH PHARMACON)
	actually recommended kits
	chemagic 360 Instrument (chemagen - PerkinElmer)
	chemagic Viral DNA/RNA Kit
	chemagic Viral NA/gDNA Kit
	QIAcube Instrument (Qiagen)
	actually recommended kits

EliGene® COVID19 UKV/SAV RT (90083-RT and 90083-RT-500) is intended for the primary detection of SARS-CoV-2 virus with simultaneous genotyping of the D80A and P681H mutations characteristic for the variant B.1.1.7 (20I/501Y.V1) also known as UK variant and B.1.351 (20H/501Y.V2) known as South African variant, respectively. The kit is complementary with the kits EliGene® COVID19 BASIC A RT (90077-RT), EliGene® COVID19 BASIC A500 RT (90077-RT-500), EliGene® COVID19 CONFIRM RT (90078-RT), EliGene® COVID19 CONFIRM 500 RT (90078-RT-500), EliGene® COVID19 Triple RIC RT (90079-RT and 90079-RT-500) and EliGene® COVID19 UKV RT (90082-RT and 90082-RT-500). Internal controls of all EliGene® kits for the detection of SARS-CoV-2 virus are identical, therefore, RNA isolated with internal control from BASIC, CONFIRM, Triple RIC or UKV kits can be analyzed by the EliGene® COVID19 UKV/SAV RT and vice versa.

RNA is recommended to be eluted in water for molecular biology. Due to the composition of the elution buffers of some manufacturers, inhibition of PCR reaction by elution buffer compounds may occur. Elution buffer of EliGene Viral RNA/DNA FAST Isolation kit can be used with no fear of PCR inhibition, as well as elution buffers of isolation kits recommended above. If you intend to use isolation kits from other manufacturers, internal control of amplification (RNA) included in this kit must be added to RNA isolation to ensure that inhibition by elution buffer is excluded.



Serum or plasma:

According to standard protocol, take the sample of serum into sterile tube. Transport samples at 4 °C to the laboratory. Serum samples are stable for a maximum of 4 days under these conditions. For longer storage, freeze the sample at -70 °C.

We recommend use volume 200 µl of serum and elution volume 50 µl of PCR water. Before the isolation, 5 µl of Internal Control RNA (IC RNA) must be added to the sample after addition of lysis buffer.

Swabs:

These specimens should be collected according to standard protocol in collection tubes.

Recommended swabs:

Flocked swabs (swabs made by the flocking technique) are inserted into the virus transport medium after swabbing.

Do not use cotton swabs due to possible inhibition of the PCR reaction. Do not use dry swabs for transport!

Samples should be transported to the laboratory at 4 °C (blue ice). Oropharyngeal and nasopharyngeal swabs are stable at 4 °C in virus transport medium for at least 3 days after sampling. For longer storage, freeze the sample at -70 °C.

Another option is to use inactivating transport media. Each inactivation medium must be validated for used RNA isolation method!

In the case of sampling in transport medium, 200 µl or quantity recommended by instruction manual of used isolation kit should be used for RNA isolation. 5µl of Internal Control RNA (IC RNA) must be added to the sample used for RNA isolation after addition of lysis buffer.

Additional required equipment

- Automatic pipettes 1-1000 µl and sterile tips with filter DNA-, RNA- free, DNase-, RNase- free (we recommended plastic with CE certificate for diagnostic purposes).
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given qPCR system. Always use only original plastic or plastics recommended by the manufacturer of the respective qPCR system. **The utilization of non-original plastic can lead to difficulties with the fluorescence readout and determination of the threshold. We cannot guarantee a correct interpretation of the results when non-original or disapproved plastics are used.**
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Equipment for qPCR – the kit is designed for qPCR instruments LightCycler® 480 (Roche; **Color compensation provided by Elisabeth Pharmacon is required!!!, please order EliGene® 4-channel Color Compensation Kit, cat. No. 90080-CC**), QuantStudio 5 (ThermoFisher Scientific), Rotor-Gene Q (Qiagen), and CFX96 (Bio-Rad). The RT-qPCR for the detection and genotyping of SARS-CoV-2 RNA utilizes TaqMan technology (FAM, HEX, Texas Red, and Cy5 probes) and can be performed on other instruments that can work with these channels.
- Lab safety gloves and respirators FFP3. Please work in appropriate biohazard boxes. Also, the centrifugation of samples must be performed in biohazard boxes. Keep in mind that also viral RNA can cause infection.
- As it is a serious pathogen, please follow actual WHO recommendations for BSL2+ or BSL3 laboratories.

Configuration of qPCR instrument

- For detection of target sequences of SARS-CoV-2, two probes labeled with FAM are used (exc. 494 nm – em. 518 nm)



- For detection of Internal control, the probe labeled with HEX is used (exc. 520 nm – em. 548 nm)
- For detection of P681H mutation, the probe labeled with TexasRed is used (exc. 589 nm – em. 615 nm)
- For detection of D80A mutation, the probe labeled with Cy5 is used (exc. 650 nm – em. 670 nm)

LightCycler® 480 (Roche):

Please, use white plates only intended for LightCycler® 480 II. The usage of natural plates can lead to decreased sensitivity of the kit. Do not reuse plates; the contamination of your laboratory could occur during the manipulation with plates.

Creation of the detection profile:

Open "Toolbox" in the "Main menu" (icon with a wrench), select "Detection formats". Select "New" detection format and assign it a name according to your choice. In the excitation and emission spectra matrix on the top right corner, click on boxes with the following combinations:

Excitation Filter	Emission Filter	Name	Melt Factor	Quant Factor	Max Integration Time
465	510	FAM	1	10	2
533	580	HEX	1	10	2
533	610	TexasRed	1	10	2
618	660	Cy5	1	10	2

In option Detection format, choose the format you have created

Set up the following temperature profile:

Step 1 - Analysis mode "None", 1 Cycle

55°C 15 min Ramp rate (4.4°C/s) Acquisition mode "None"

Step 2 - Analysis mode "None", 1 Cycle

95°C 2 min Ramp rate (4.4°C/s) Acquisition mode "None"

Step 2 - Analysis mode "Quantification", 45 Cycles

95°C 5 s Ramp rate (4.4°C/s) Acquisition mode "None"

55°C 15 s Ramp rate (2.2°C/s) Acquisition mode "Single"

67°C 15 s Ramp rate (4.4°C/s) Acquisition mode "None"

Step 3 - Analysis mode "None", 1 Cycle

40°C 20 s Ramp rate (2.2°C/s) Acquisition mode "None"

The complete temperature profile can be uploaded from Run Template "EliGene COVID19 UKV/SAV RT_LC480.ixo". The Run Template can be imported to the software in the menu "Navigator" by clicking to icon "Import" from the CD included in the kit.

QuantStudio 5 (ThermoFisher Scientific):

Use the Experiment type, "Presence/Absence", Chemistry "TaqMan Probes", and Run Mode "Standard". As a reporter, dyes use FAM (SARS-Cov2), HEX (IC RNA), ROX (P681H) and Cy5 (D80A). **Do not use any passive reference dye!**

Set up the following temperature profile:

Holding stage



55°C 15 min Ramp rate (1.6°C/s)

Holding stage

95°C 2 min Ramp rate (1.6°C/s)

Cycling stage – 45 cycles

95°C 5 s Ramp rate (1.6°C/s)

55°C 15 s Ramp rate (1.6°C/s) Data collection ON

67°C 15 s Ramp rate (1.6°C/s)

Post-Read Stage

40°C 20 s Ramp rate (1.6°C/s)

Collect the emission signal at the second step at 55 °C

The complete temperature profile can be uploaded from Run Template "EliGene COVID19 UKV/SAV RT_QS3.edt" or "EliGene COVID19 Triple RIC RT_QS5.edt". The Run Template can be copied from the CD included in the kit.

RotorGene Q (Qiagen):

In the "New Run" window, choose "Three Step" run

Choose the appropriate "Rotor Type" and click "Next".

Set up the following temperature profile:

Holding stage

55°C 15 min

Holding stage

95°C 2 min

Cycling stage – 45 cycles

95°C 5 s

55°C 15 s Acquiring in channels "Green", "Yellow", "Orange" and "Red"

67°C 15 s

Holding stage

40°C 20 s

For the Gain optimization in all channels, select the option "Automatic gain optimization before first acquisition". The complete temperature profile can be uploaded from Run Template "EliGene COVID19 UKV/SAV RT_Q-GENE.ret". The Run Template can be copied from the CD included in the kit.

CFX96 Touch (Bio-Rad):

In Startup Wizard Create a new Experiment for CFX96 instrument and Create New Protocol.

Set up the following temperature profile:

Step 1 55°C 15 min

Step 2 95°C 2 min

Step 3 95°C 5 s

Step 4 55°C 15 s + Plate Read

Step 5 67°C 15 s

Step 6 GOTO Step 3 44x



Step 7 40°C 20 s

Enter the Sample Volume 20ul

Collect the emission signal at Step 4 at 55° C.

For filter settings, use the "Scan Mode" All Channels, and in Plate Manager, select for the samples only fluorophores FAM, HEX, TexasRed, and Cy5. Then assign the samples with positions and Targets as an Unknown sample (Samples) or Standard. The complete temperature profile can be uploaded from Run Template "EliGene COVID19 UKV/SAV RT_CFX.pcr". The Run Template can be copied from the CD included in the kit.

Reagent preparation

- To avoid contamination, keep all tubes closed and follow the instructions.
- All reagents must be completely thawed before the usage, briefly mixed on vortex, and shortly spun.
- Add 5 µl of Internal Control RNA (IC RNA) to sample with lysis buffer. Never add Internal Control RNA to isolated RNA before starting PCR!

WARNING: The contamination in laboratory space is possible. Use separate pipette for Master Mixes, separate pipette for positive controls, and separate pipette for samples! Follow all recommendations for laboratories providing RNA analyses.

Preparation of Master Mix

1. Take the CoV USV Mix tube and the Enzyme Mix tube, and then thaw at room temperature. Immediately after thawing, spin briefly in a centrifuge. Prepare the Master Mix by mixing 14 µl CoV USV Mix and 1 µl Enzyme Mix per reaction and spin briefly.
2. Detection: Add 15 µl of the Master Mix to the amplification tubes or plates and add 5 µl of the isolated RNA sample. Be careful when pipetting the sample to avoid cross-contamination of the samples. The prepared Master Mix should be used within 30 minutes and cannot be reused. Do not freeze prepared Master Mix.
3. Positive Control: Pipette 15 µl of the Master Mix separately into the amplification tube or plate. Then add 5 µl of PC RNA CoV USV. Be careful when pipetting the positive control to avoid contamination of samples. **Use a different micropipette for pipetting, only positive controls.**

Insert the microtubes or plate into the qPCR instrument and run the program as described in Configuring the qPCR Instrument above.

Result reading

LightCycler[®] 480 (Roche):

In the "Sample Editor" menu, choose "Abs Quant" workflow.

In the menu, "Analysis" chooses "Abs Quant/2nd Derivative Max" option.

It is compulsory to perform the analysis of the data with active Color Compensation. Otherwise, data from HEX and Texas Red channel could not be interpreted. Select a Color Compensation file for FAM, HEX, TexasRed, and Cy5.

The positive result for SARS-CoV-2: The positive result is characterized by the growth of the fluorescence signal in the FAM channel (465-510). In a case of negative results, the amplification will not occur.

The positive result for P681H mutation: The positive result is characterized by the growth of fluorescence signal in the TexasRed channel (533-610). In a case of negative results, the amplification will not occur.



The positive result for D80A mutation: The positive result is characterized by the growth of fluorescence signal in the Cy5 channel (618-660). In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by a growth of signal in the HEX channel (533-580).

QuantStudio 5 (ThermoFisher Scientific):

In "Analyse Settings" choose "Automatic Threshold" and "Automatic Baseline" option and analyze results.

The positive result for SARS-CoV-2: The positive result is characterized by the growth of the fluorescence signal in the FAM channel. In a case of negative results, the amplification will not occur.

The positive result for P681H mutation: The positive result is characterized by the growth of the fluorescence signal in the TexasRed (ROX) channel. In a case of negative results, the amplification will not occur.

The positive result for D80A mutation: The positive result is characterized by the growth of fluorescence signal in the Cy5 channel. In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of the fluorescence signal in the HEX (VIC) channel.

Rotor-Gene Q (Qiagen):

Click to "Analysis" icon in the menu and choose the Analysis option "Quantitation". In the "Quantitation Analysis" window, select "Dynamic Tube" and "Slope Correct" option. In the option "Outlier Removal" setup NTC Threshold value for 10 %.

The positive result for SARS-CoV-2: The positive result is characterized by the growth of the fluorescence signal in the FAM channel (Green). In a case of negative results, the amplification will not occur.

The positive result for P681H mutation: The positive result is characterized by the growth of the fluorescence signal in the TexasRed channel (Orange). In a case of negative results, the amplification will not occur.

The positive result for D80A mutation: The positive result is characterized by the growth of fluorescence signal in the Cy5 channel (Red). In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of the fluorescence signal in the HEX channel (Yellow).

CFX96 Touch (Bio-Rad):

In the Data Analysis window, choose "Quantification". In "Settings" menu, choose "Baseline Setting" option, and select "Baseline Subtracted Curve Fit" and "Apply Fluorescence Drift Correction".

In the Data Analysis window, select a single fluorophore (FAM, HEX, TexasRed, Cy5) by clicking the box next to the fluorophore name located under the amplification chart and read the results for individual samples.

The positive result for SARS-CoV-2: In "Settings" select "Baseline Threshold" and set the "Single Threshold" baseline to "Auto Calculated". The positive result is characterized by the growth of the fluorescence signal in the FAM channel. In a case of negative results, the amplification will not occur.

The positive result for P681H mutation: In "Settings" select "Baseline Threshold" and set the "Single Threshold" baseline to "Auto Calculated". The positive result is characterized by the growth of the fluorescence signal in the TexasRed channel. In a case of negative results, the amplification will not occur.

The positive result for D80A mutation: In "Settings" select "Baseline Threshold" and set the "Single Threshold" baseline to "Auto Calculated". The positive result is characterized by the growth of the fluorescence signal in the Cy5 channel. In a case of negative results, the amplification will not occur.



The Internal Control must be amplified in each sample. In "Settings" select "Baseline Threshold" and set the "Single Threshold" baseline to "Auto Calculated". The Internal Control amplification is characterized by the growth of the fluorescence signal in the HEX channel.

Interpretation of results

Negative result:

If the increase of amplification signal in FAM channel does not appear before cycle number 40 with appropriate threshold settings applied, the result of the test should be interpreted as probably negative or with a concentration of RNA below the detection limit of this kit (15 genomic RNA/reaction). The signal for Internal Control must be positive – see article Quality control. Positive signals in Texas Red and Cy5 channels themselves do not mean presence of SARS-CoV-2 virus in the sample if there is no signal in FAM channel simultaneously. **For weakly positive specimens where the Ct is greater than 30 in the FAM channel for coronavirus detection, positivity for mutations may not be correctly read.**

This result does not exclude the occurrence of SARS-CoV-2 and determination of D80A and P681H mutation presence in a sample because the results of this test are dependent on proper sample collection and processing. Results are also dependent on an adequate quantity of analyzed RNA. **It has been reported that the viruses can be secreted intermittently, and even in an infected patient, the virus level in clinical specimens may be below the detection limit of any RT-qPCR method in each day. For this reason, it is recommended to perform at least two, ideally more RT-qPCR examinations in a single patient over several days.**

Positive result:

If the amplification signal in FAM, TexasRed, and Cy5 channel appears before cycle number 40 at the appropriate threshold baseline, RNA of the respective virus is detected in the sample. **It is absolutely essential to evaluate signal in each channel separately! The fluorescence signal in the FAM channel is stronger than in remaining channels! The signal in Texas Red and Cy5 channels for the must be evaluated with regard to the FAM channel. The difference between cycle number values between FAM and Texas Red/Cy5 channels should be no more than 3.**

It must be noted that the amplification signal for both mutations in Texas Red and Cy5 channels can be indistinguishable from the background in weakly positive samples (Cycle number higher than 30) even if the mutation(s) is present. The cycle number values in Texas Red and Cy5 channels must lie within 3 cycle difference, if both mutations are present. Higher difference means inconclusive result.

IMPORTANT!

Due to the rapid evolution of the SARS-CoV-2 virus, new mutation can occur in time and the definition of the variants can change. We advise users to check the newest information about the SARS-CoV-2 classification in order to prevent possible misclassification of the SARS-CoV-2 virus. It is strongly recommended to perform sequencing of samples in particular time and area (whole genome or just part of the SARS-CoV-2 virus genome) in order to identify the SARS-CoV-2 virus variant circulating in the area precisely!

This SARS-CoV-2 virus detection and genotyping kit is intended for the routine screening of large sets of samples and does not serve for the full genotyping of the SARS-CoV-2 virus variants. The usage of this kit



does not replace sequencing.

Interpretation results table

SARS-CoV-2 FAM channel	P681H Texas Red channel	D80A Cy5 channel	Interpretation
Positive	Positive	Negative	Variant B.1.1.7 (20I/501Y.V1) detected
Positive	Negative	Positive	Variant B.1.351 (20H/501Y.V2) detected
Positive	Negative	Negative	None of the variants detected*
Positive (>30)	Negative	Negative	Variants can not be determined
Positive (<30)	Negative	Negative	None of the variants detected*

* Lack of the D80A or P681H presence can be a sign of the new variant. It is recommended to perform sequencing to confirm the absence of D80A or P681H and identify other clinically important mutations.

Inhibited sample:

In the case that increase of the amplification signal is observed in none of the channels, including the HEX channel for the IAC, the analysis should be repeated, preferably with newly isolated RNA samples. Make sure the elution buffer does not inhibit the qPCR reaction. In this case, it is recommended to perform elution into the water for molecular biology.

Control procedure

EliGene® COVID19 UKV/SAV RT kit involves Internal Control. Internal Control follows the quality of RNA isolation and detects the inhibition of reverse transcription and amplification. The internal control must be added directly to the sample with lysis buffer before the viral RNA isolation. In the case that no amplification occurs in FAM, TexasRed, and Cy5 channels, there must be present an amplification in the HEX channel (internal amplification control) with cycle number value lower than 36.

Additionally, **cycle number values of all the samples undergoing the same procedure of RNA isolation must have cycle number value of IAC in the HEX channel within the range of 3 cycles.** The higher fluctuation of the cycle number values in a single qPCR run indicates non-standard conditions in the RNA isolation. However, this condition does not apply to highly positive samples (cycle number in FAM channel < 20).

Reference material:

To monitor the whole examination process covering RNA isolation and qPCR detection is possible to use reference viral material positive for SARS-CoV-2. The positive commercial material is not available. Do not use artificial RNA or DNA or positive controls from other manufacturers.

Troubleshooting:

1. If there is no amplification of Internal Control, there is some problem in the isolation of RNA or the kit is after the expiration date or there is qPCR instrument malfunction.



2. If the cycle number range of the Internal Amplification Control is higher than 3 cycles, there is a non-homogeneity in the RNA isolation cause probably by the partial inhibition of the qPCR. Repetition of the RNA isolation should be considered in this case. Another possibility is to dilute the isolated RNA twice, alternatively more times.
3. If there is no amplification of Positive Control, the kit is after the expiration date or there is qPCR instrument malfunction. It may also be a failure to follow the recommended procedure for sample preparation and analysis.

Performance characteristics

Analytical performance characteristics:

Analytical sensitivity of EliGene® COVID19 UKV/SAV RT kit defined as the lowest number of genomic RNA copies present in the amplification reaction that can be successfully amplified in 3 independent repeats is 5 genomic RNA of SARS-CoV-2 added in Master Mix. The sensitivity of the RT-qPCR procedure depends on the method of RNA isolation. The sensitivity of the method was verified as follows. There were prepared dilution series of positive control of known concentration. They were tested 3 times. The SARS-CoV-2 detection was 100% successful in all the samples, which contain 5 or 50 and more RNA in Master Mix.

Analytical sensitivity is 5 copies of SARS-CoV-2 in reaction Mix.

The analytical specificity of the method is 100%. All the primers and probes were adopted from the literature and/or approved by the authorities like WHO, CDC, or ECDC. Additionally, the method's analytical specificity was analyzed by comparison of primers and probes sequences with all known RNA and DNA sequences in the GenBank database, and no cross-reaction was found. No cross-reaction with the human genome was found.

Clinical specificity and sensitivity were tested on 3439 clinical specimens SARS-CoV-2. As reference material samples, combination of oropharyngeal and nasopharyngeal swabs were used. Samples were independently tested by EliGene® COVID19 UKV/SAV RT kit and by reference methods – by CE IVD EliGene® COVID19 CONFIRM RT.

Sensitivity and specificity of SARS-CoV-2 detection

A = 553 Really positive	B = 0 False positive
C = 0 False negative	D = 2886 Really negative

$$\text{Sensitivity} = A/(A+C) = 553/(553+0) = 100\%$$

$$\text{Specificity} = D/(D+B) = 2886/(2886+0) = 100\%$$

Clinical sensitivity and specificity of EliGene® COVID19 UKV RT kit for SARS-CoV-2 is 100%.

The specificity and sensitivity of mutation detection was determined by comparing the results from Spike protein sequencing on twenty six samples with the results of detection with the EliGene® COVID19 UKV RT kit. Agreement was 100% for all samples with a Ct value less than 30.

Specificity of mutations detection

Sample	Sequencing		EliGene® COVID19 UKV/SAV RT	
	D80A	P681H	D80A	P681H
A2	WT	WT	Negative	Negative



A3	WT	WT	Negative	Negative
B2	WT	WT	Negative	Negative
B3	WT	Mutation	Negative	Positive
B4	WT	WT	Negative	Negative
C2	WT	WT	Negative	Negative
C3	WT	WT	Negative	Negative
C4	WT	Mutation	Negative	Positive
D2	WT	Mutation	Negative	Positive
D3	WT	WT	Negative	Negative
D4	WT	Mutation	Negative	Positive
E2	WT	Mutation	Negative	Positive
E3	WT	Mutation	Negative	Positive
E4	WT	WT	Negative	Negative
F2	WT	WT	Negative	Negative
F3	WT	WT	Negative	Negative
G2	WT	WT	Negative	Negative
G3	WT	Mutation	Negative	Positive
H2	WT	Mutation	Negative	Positive
H3	WT	Mutation	negative	positive
A4	Mutation	WT	Positive	Negative
A5	Mutation	WT	Positive	Negative
A6	Mutation	WT	Positive	Negative
A7	Mutation	WT	Positive	Negative
A8	Mutation	WT	Positive	Negative
A9	Mutation	WT	Positive	Negative

Analytical specificity and sensitivity for the detection of **D80A** and **P681H** mutations is 100% in samples with a Ct value below 30.

Diagnostic performance characteristics:

Measuring interval

The kit enables the detection of 5×10^0 - 5×10^8 of viral RNA molecules in Reaction Mix.

Internal control of quality

As an internal control of quality, the Internal Control (IC RNA) for checking the process of RNA isolation, reverse transcription, and DNA amplification is used. Positive control for functional control of Master Mix and as a reference sample is used.

Limitation of the examination procedure

The sensitivity of the kit depends on handling the specimen (isolation of RNA). It is strictly recommended to use isolation kits and procedures recommended in this manual.

A negative result does not exclude the occurrence of viral infection. The results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed RNA. The presence of any of the viruses detected RNA in clinical samples of infected persons is dependent on the infection phase and could be intermittent. The attending physician must give a conclusion on the diagnosis and treatment of patients.

Biological reference intervals



Not applicable information for this kit.

Warning

After the preparation, the Master Mix is stable for 30 minutes. Do not freeze tubes with Master Mix repeatedly! Do not mix components of the kits of different lots!

Warnings and general precautions

This kit is intended for *in vitro* use only.

- Lab safety gloves and respirators FFP3 are necessary for work. Please work in appropriate biohazard boxes. Also centrifugation of samples must be performed in biohazard boxes. Keep in mind that also viral RNA can cause infection.
- **As SARS-CoV-2 is a serious pathogen, please follow actual WHO recommendations for BSL2+ or BSL3 laboratories!**
- Handle and dispose of all biological samples as if they could transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121 °C for one hour before disposal.
- Handle and dispose of all reagents and all assay materials as if they could transmit infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.
- Wear suitable protective clothing and gloves and protect eyes/face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Wash hands carefully after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with regulations in force.
- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.
- Do not use reagents from other manufacturer's kit.
- Do not change recommended protocol for PCR analysis!

Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.
- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection



of amplification products to the area designed for the extraction/preparation of the amplification reactions.

- The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Reagents must be handled in PCR box. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

Warnings and precautions specific to components of the kit

The tubes containing CoV UKV Mix and Enzyme Mix are disposable and therefore must be used once only in the preparation of the reaction mixture.

These Mixes carry the following safety warnings (P):

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P281 Use personal protective equipment as required.

The tubes containing IC RNA are disposable and therefore must be used once only in the preparation of the reaction mixture.

In case of any problems, please contact ELISABETH PHARMACON, Ltd.

Literature

Ashour HM, Elkhatab WF, Rahman MM, Elshabrawy HA. Insights into the Recent 2019 Novel Coronavirus (SARS-CoV-2) in Light of Past Human Coronavirus Outbreaks. *Pathogens*. 2020 Mar 4;9(3). pii: E186. doi: 10.3390/pathogens9030186.

He F, Deng Y, Li W. Coronavirus Disease 2019 (COVID-19): What we know? *J Med Virol*. 2020 Mar 14. doi: 10.1002/jmv.25766.

Khan S, Siddique R, Shereen MA, Ali A, Liu J, Bai Q, Bashir N, Xue M. The emergence of a novel coronavirus (SARS-CoV-2), their biology and therapeutic options. *J Clin Microbiol*. 2020 Mar 11. pii: JCM.00187-20. doi: 10.1128/JCM.00187-20.

Maison DP, Ching LL, Shikuma CM, Nerurkar VR. Genetic Characteristics and Phylogeny of 969-bp S Gene Sequence of SARS-CoV-2 from Hawaii Reveals the Worldwide Emerging P681H Mutation. *bioRxiv*. 2021 Jan 7:2021.01.06.425497. doi: 10.1101/2021.01.06.425497. PMID: 33442699; PMCID: PMC7805472.



Symbols



Catalog number



Upper limit of temperature



Batch code



Use by (last day of month)



in vitro diagnostic medical device



Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.



Contains sufficient for "N" tests



Attention, consult instructions for use



Manufacturer

Manufacturer

ELISABETH PHARMACON Ltd.

Rokycanova 4437/5, 615 00 Brno, Czech Republic Tel.: +420 542 213 851, +420 542 213 827

E-mail: info@elisabeth.cz