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EliGene® COVID19 BASIC A RT

REF 90077-RT-A (for 100 samples)

Kit components:

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

Storage and shelf life after first opening:

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 BASIC A RT kit is intended for qualitative RNA detection of SARS-CoV-2 virus.

Principle of the method

This diagnostic kit is based on reverse transcription of SARS-CoV-2 viral RNA and subsequent one-step qPCR analysis. Innovative mixture of 8 sets of primers and 4 TaqMan probes (FAM labeled probes for virus detection and HEX labeled probe for internal control visualization) mixed in the ready-to-use SARS-CoV-2 Mix is used. Primers and probes utilized in the kit were approved and recommended by WHO. Increased sensitivity and specificity of this kit is based on the amplification of 3 independent SARS-CoV-2 targets in a single PCR 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 family *Coronaviridae* that is the largest family within the order *Nidovirales*. SARS-CoV-2 is spherical positive single-stranded RNA virus that is characterized by spike proteins projecting from the virion surface. It is 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). The RNA genome of coronaviruses is the second largest of all RNA viruses, SARS-CoV-2 has 29,9 kilobases in size.

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
serum, plasma	chemagic Viral DNA/RNA Kit (chemagen-PerkinElmer)
	QIAamp Virus Spin Kit or kits recommended by Qiagen

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Automatic isolation:

ZEPHYRUS Magneto (ELISABETH PHARMACON)
MAGNETO BodyFluid DNA/RNA Isolation Kit

chemagic 360 Instrument (chemagen-PerkinElmer)
chemagic Viral DNA/RNA Kit
chemagic Viral NA/gDNA Kit

QIAcube Instrument (Qiagen)
kits recommended by Qiagen

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 or plasma into sterile tubes.

We recommend use volume 200 µl of serum or plasma 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:

FLOQSwabs (Copan) – dry swabs or in UTM - Universal Transport Medium (Copan)

Dacron swabs – dry or medium for virus transport MicroTest™ M4RT or MicroTest™ M6 (Thermo Scientific)

Other polymer fiber collection kits – dry or with transport medium for viruses.

Do not use cotton swabs that can inhibit PCR.

Specimens should be transported to the laboratory at 4 °C (blue ice). They are stable minimally 72 hours from sampling at 4°C. In the case, that you have no possibility to transport dry swabs to laboratory at 4°C, at room temperature dry swabs should be transported until 6 hours.

For storage of samples longer than 72 hours, freeze sample to -20 °C.

Dry swabs should be submerged in lysis buffer according to instruction manual of used isolation kit. After swab removal, **5µl of Internal Control RNA (IC RNA) must be added to the sample used for RNA isolation after addition of lysis buffer.**

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 RealTime PCR system.
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Equipment for RealTime PCR – the kit is designed for RealTime Systems LightCycler® 480, QuantStudio 3 and 5 Real-Time PCR Systems (ThermoFisher Scientific), Rotor-Gene Q (Qiagen) and Real-Time PCR system CFX96 (Bio-Rad). The RT-qPCR for the detection of SARS-CoV-2 RNA utilizes TaqMan technology (FAM and HEX probes) and can be performed on other instruments that can work in FAM and HEX channels
- Lab safety gloves and respirators FFP3. 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 it is a serious pathogen, please follow actual WHO recommendations for BSL2+ or BSL3 laboratories.

Configuration of Real Time instrument

- For detection of target sequences of SARS-CoV-2 three 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)

LightCycler® 480 (Roche):

Please, use white plates only intended for LightCycler® 480. 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.

In option Detection format choose “2 Color Hydrolysis probe”.

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 up-loaded from Run Template “EliGene COVID19 BASIC A_LC480.ixo”. The Run Template can be imported to the software in menu “Navigator” by clicking to icon “Import” from the CD included in the kit.

QuantStudio 3 and 5 Real-Time PCR Systems (ThermoFisher Scientific):

Use the Experiment type,” Presence/Absence”, Chemistry “TaqMan Probes”, and Run Mode “Standard”.

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 emission signal at the second step at 55 °C

The complete temperature profile can be up-loaded from Run Template "EliGene COVID19 BASIC A RT_QS3.edt" or "EliGene COVID19 BASIC A RT_QS5.edt". The Run Template can be copied from the CD included in the kit.

RotorGene 6000 or 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" and "Yellow"

67°C 15 s

Holding stage

40°C 20 s

For the Gain optimization in all channels select option "Automatic gain optimization before first acquisition". The complete temperature profile can be up-loaded from Run Template "EliGene COVID19 BASIC A RT_Q-GENE.ret". The Run Template can be copied from the CD included in the kit.

CFX96 Touch Real-Time PCR Detection System (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 emission signal at the Step 4 at 55° C.

For filter settings use the "Scan Mode" All Channels but in Plate Manager select for the samples only fluorophores FAM and HEX. Then assign the samples with positions and Targets FAM and HEX as an Unknown sample (Samples) or Standard.

Reagent preparation

- To avoid the contamination, keep all tubes closed and follow the instructions.
- Before the usage, all reagents must be completely thawed, 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 also 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 SARS-CoV-2 Mix tube and the Enzyme Mix tube and thaw at room temperature. Immediately after thawing, spin briefly in centrifuge. Prepare the Master Mix by mixing 14 µl SARS-CoV-2 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 5µl of PC RNA SARS-CoV-2. 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 RealTime PCR instrument and run the program as described in Configuring the RealTime PCR Instrument above.

Result reading

LightCycler® 480 (Roche):

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

In menu "Analysis" choose "Abs Quant/2nd Derivative Max" option.

Positive result for SARS-CoV-2: The positive result is characterized by the growth of fluorescence signal in FAM channel (465-510). 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 HEX channel (533-580).

QuantStudio 3 and 5 Real-Time PCR Systems (ThermoFisher Scientific):

In “Analyse Settings” choose “Automatic Treshold” and “Automatic Baseline” option and analyze results.

Positive result for SARS-CoV-2: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm). 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 fluorescence signal in HEX channel (em. 548 nm).

Rotor-Gene Q (Qiagen):

Click to “Analysis” icon in the menu and choose Analysis option “Quantitation”. In “Quantitation Analysis” window choose “Dynamic Tube” and “Slope Correct” option.

Positive result for SARS-CoV-2: The positive result is characterized by the growth of fluorescence signal in FAM channel (Green). 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 fluorescence signal in HEX channel (Yellow).

The values of “Calc. conc.” correspond to the quantity of positive result; “Negative” means negative result.

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Data Analysis window choose “Quantification”. In “Settings” menu choose option “Baseline Threshold” and select “Baseline Cycles” option as “Auto Calculated” and Single “Threshold” option as “Auto Calculated”.

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

Positive result for SARS-CoV-2: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm). 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 fluorescence signal in HEX channel (em. 548 nm).

Interpretation of results

Negative result:

If the increase of amplification signal in FAM channel does not appear before cycle number 40, the result of test should be interpreted as probably negative or with 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. This result does not exclude the occurrence of SARS-CoV-2 infection because results of this test are dependent on proper sample collection and processing. Results are also dependent on adequate quantity of analyzed SARS-CoV-2 RNA. **It has been reported that the virus 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 given days. 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:

Amplification signal in FAM channel appears before cycle number 40. SARS-CoV-2 RNA was detected in the sample. The sample is SARS-CoV-2 RNA positive.

Inhibited sample:

In the case that increase of the amplification signal specific for SARS-CoV-2 in FAM channel and also increase the of amplification signal specific for internal control in HEX channel is not observed,

the analysis should be repeated preferably with newly isolated RNA samples. Make sure the elution buffer does not inhibit the PCR reaction. In this case it is recommended to perform elution into water for molecular biology.

Control procedure

EliGene® COVID19 BASIC A RT kit involves Internal Control. Internal Control follows the quality of RNA isolation and detects the occurrence of an inhibition of reverse transcription and amplification process. In the case that the sample is SARS-CoV-2 RNA negative, Ct of Internal Control must be Ct < 40. The Internal Control must be added to the sample before the RNA isolation. The internal control must be added directly to the sample with lysis buffer prior to commencing isolation of the viral RNA.

Reference material:

To monitor the all examination process covering RNA isolation and RealTime PCR detection is possible to use reference viral material positive for SARS-CoV-2. The commercial positive 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 RealTime instrument breakdown.
2. If there is no amplification of Positive Control, the kit is after the expiration date or there is RealTime instrument breakdown. 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 BASIC A RT kit is 15 genomic RNA added in Master Mix. Sensitivity of PCR procedure depends on the method of RNA isolation. The sensitivity of method was verified as follows. There were prepared dilution series of positive RNA samples of known concentration. Totally it was tested for three times. The SARS-CoV-2 detection was 100% successful in all the samples which contain 15 and more RNA in reaction Mix.

Analytical sensitivity is 15 copies of SARS-CoV-2 RNAs in reaction Mix.

Analytical specificity of method is 100%. All the primers and probes were approved and recommended by WHO. Additionally, analytical specificity of method was analysed by comparison of primers and probes sequences with all known RNA and DNA sequences in GenBank database and no cross reaction was found. No cross reaction with human genome was found.

Clinical specificity and sensitivity was tested on 100 clinical specimens. As reference material samples, nasopharyngeal swabs were used. Samples were independently tested by EliGene® COVID19 BASIC A RT kit and by two reference methods - by one different method recommended by WHO and by CE IVD marked kit of competitive manufacturer.
EliGene® COVID19 BASIC A RT kit showed In comparison with two reference methods 100% agreement:

Really positive (A) = 18
False positive (B) = 0
False negative (C) = 0
Really negative (D) = 82

Sensitivity = A/(A+C) = 18/(18+0) = 100%
Specificity = D/(D+B) = 82/(82+0) = 100%

Clinical sensitivity and specificity of EliGene® COVID19 BASIC A RT kit is 100%.

Diagnostic performance characteristics:

Measuring interval

The kit enables the detection of 1.5×10^{1} - 1.5×10^{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 kit depends on handling with specimen (isolation of RNA). It is strictly recommended to use isolation kits and procedures recommended in this manual.
Negative result does not exclude the occurrence of SARS-CoV-2 infection. Results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analysed SARS-CoV-2 RNA. The presence of SARS-CoV-2 RNA in clinical samples of infected persons is dependent on infection phase and could be intermittent. The final conclusion on the diagnosis and treatment of patients must be given by the attending physician.

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 SARS-CoV-2 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

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.

Ashour HM, Elkhatib 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.

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

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