13909197-1 (en)

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3501-0010 (96 reactions)

SARS-CoV-2 RT-qPCR Reagent kit

Real-time reverse transcriptase polymerase chain reaction

Instructions for use.

Manufacturer:
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Mustionkatu 6, FI-20750 Turku, Finland
www.perkinelmer.com

FOR *IN VITRO* DIAGNOSTIC USE

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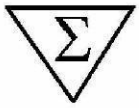
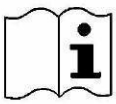
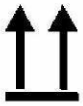
PerkinElmer®

SYMBOLS

Symbol	Symbol Title and Reference Number	Symbol Source / Standard Title and Number	Description
IVD	*In vitro* diagnostic medical device No. 5.5.1	ISO 15223, Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied	Indicates a medical device that is intended to be used as an *in vitro* diagnostic medical device.
LOT	Batch code No. 5.1.5	ISO 15223, Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied	Indicates the manufacturer's batch code so that the batch or lot can be identified.
PN	Packing number	Not applicable	Not applicable
REF	Catalogue number No. 5.1.6	ISO 15223, Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	Use-by date No. 5.1.4	ISO 15223, Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied	Indicates the date after which the medical device is not to be used.
	Temperature limit No. 5.3.7	ISO 15223, Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied	Indicates the temperature limits to which the medical device can be safely exposed.
	Keep away from sunlight / Keep away from heat No. 5.3.2	ISO 15223, Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied	Indicates a medical device that needs protection from light sources.

Symbol	Symbol Title and Reference Number	Symbol Source / Standard Title and Number	Description
	Contains sufficient for <n> tests No. 5.5.5	ISO 15223, Medical Devices – Symbols to be used with medical device labels, labeling and information to be supplied	Indicates the total number of IVD tests that can be performed with the IVD kit reagents.
	Consult instructions for use No. 5.4.3	ISO 15223, Medical Devices – Symbols to be used with medical device labels, labeling and information to be supplied	Indicates the need for the user to consult the instructions for use.
	Manufacturer No. 5.1.1	ISO 15223, Medical Devices – Symbols to be used with medical device labels, labeling and information to be supplied	Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
	This way up	IATA (International Air Transport Association)	Not applicable
	Recyclable	RESY Organization für Wertstoffentsorgung GmbH	Not applicable

SARS-CoV-2 RT-qPCR Reagent kit

INTENDED USE

The kit is intended for the qualitative detection of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) nucleic acids in RNA extracted from the human oropharyngeal swab and nasopharyngeal swab specimens as an aid in diagnosing patients suspected of COVID-19 (coronavirus disease) by their healthcare provider. Clinical correlation with patient history and other diagnostic information is necessary to determine the patient's infection status.

SUMMARY AND EXPLANATION OF THE ASSAY

SARS-CoV-2 RNA is generally detectable in human oropharyngeal swab and nasopharyngeal swab specimens during the acute phase of SARS-CoV-2 virus infection [1]. Positive results are indicative of presence of SARS-CoV-2 RNA. However, positive results do not rule out bacterial infection or co-infection with other viruses. Negative results do not exclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The SARS-CoV-2 RT-qPCR Reagent kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

PRINCIPLES OF THE ASSAY

The SARS-CoV-2 Real-time RT-PCR assay uses TaqMan™-based real-time PCR technique to conduct in vitro transcription of SARS-CoV-2 RNA, DNA amplification and fluorescence detection.

The assay targets at the specific genomic regions of SARS-CoV-2: nucleocapsid (N) gene and ORF1ab [2]. The TaqMan™ probes for the two amplicons are labeled with FAM™ and HEX™ /VIC™ fluorescent dyes, respectively, to generate target-specific signal.

The assay includes probes for human RNA target that is used as an RNA internal control to monitor the processes from nucleic acid extraction to fluorescence detection. The Internal Control (IC) probe is labeled with Cy5® fluorescent dye to differentiate its fluorescent signal from SARS-CoV-2 targets. The assay also uses a dUTP/UNG carryover prevention system to avoid contamination of PCR products and subsequent false positive results.

TaqMan is a trademark of Roche Molecular Systems, Inc.
FAM and VIC are trademarks of Thermo Fisher Scientific.
HEX and QuantStudio are trademarks of Thermo Fisher Scientific.
Cy5 is a registered trademark of GE Healthcare UK Limited.

KIT CONTENTS

Each 3501-0010 SARS-CoV-2 RT-qPCR Reagent kit contains reagents for 96 reactions that can be used in maximum of four separate runs.

The expiry date of the unopened kit is stated on the outer label. Store the kit at -30 – -16 °C. Thaw the Enzyme mix on ice or at +2 – +6 °C. Allow the other reagents to thaw for 30 minutes to bring them to room temperature (+19–+25 °C) before use. The kit can tolerate up to four freeze and thaw cycles. Once opened, the kit should be used within 30 days.

Reagents

Component	Quantity	Shelf life and storage
CoV2 Reagent A Buffers, Mg^{2+}, primers, probes	1 vial, 110 µL	-30 – -16 °C protected from light until expiry date stated on the vial label.
CoV2 Enzyme Mix DNA polymerase, MMLV Reverse transcriptase, dNTPs, RNase inhibitor, UNG/dUTP	1 vial, 550 µL	-30 – -16 °C until expiry date stated on the vial label.
CoV2 Positive Control SARS-CoV-2 RNA fragments in plasmids	1 vial, 70 µL	-30 – -16 °C until expiry date stated on the vial label.
CoV2 Negative Control Nuclease-free water	1 vial, 1000 µL	-30 – -16 °C until expiry date stated on the vial label.
Lot-specific quality control certificate	1 pc	

MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT

The following items are required and available from Wallac Oy or PerkinElmer, Inc. and its distributors.

1. RNA extraction: It is recommended to use PerkinElmer nucleic acid extraction kits (e.g. CMG-1033-S) with the chemagic™ 360 instrument and software. Other equivalent RNA extraction system may be used.

In addition, the following are required:

- Real-time RT-PCR: Instruments with FAM™, HEX™/VIC™ and Cy5® channels (e.g. Roche Life Science LightCycler® 480 System, Thermo Fisher QuantStudio™, Bio-Rad CFX96 Real Time System)
- Aerosol resistant barrier pipette tips (for volumes 1–100 µL, 1–200 µL, 100–1000 µL, 1000–5000 µL)
- Full-skirted 96-well PCR plates. The footprint and well spacing of the plates should meet ANSI-SBS standards 1-2004 and 4-2004, and the plates should be certified free of DNase, RNase, and human DNA.
- Clear adhesive PCR seals for 96-well PCR plates
- Pipettors (for volumes 1–100 µL, 1–200 µL, 100–1000 µL, 1000–5000 µL)
- Multichannel pipettor (either 12-channel or 8-channel pipettor for dispensing 10 µL and 20 µL)
- Vortex mixer
- Microcentrifuge capable of spinning down liquid in 1.5 mL microcentrifuge tubes
- Adhesive seal/film applicator (e.g. Thermo Fisher Scientific Inc., Hampton Research Corp., or Life Technologies Corporation)
- Plate centrifuge capable of centrifuging 96-well PCR plates at 1600 x *g* for 1 min (e.g. Labnet International, Inc.)
- Silicone spacer plate or compression pad for 96-well plate (e.g. Sigma Aldrich, Inc. or Life Technologies Corporation)

SPECIMEN COLLECTION AND HANDLING

Purified RNA is used as the template for SARS-CoV-2-specific Real-time reverse transcriptase polymerase chain reaction described in the assay procedure section.

For RNA extraction it is recommended to use PerkinElmer nucleic acid extraction kits (e.g. CMG-1033-S) with the chemagic™ 360 system or equivalent RNA extraction method.

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

This kit should only be used by adequately trained personnel.

The use of this kit should be strictly in accordance with the nucleic acid amplification guidelines to operate in compliance with the requirements of the appropriate laboratories.

Handle all patient specimens as potentially infectious.

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chemagic is a trademark of PerkinElmer chemagen Technologie GmbH.
LightCycler is a registered trademark of Roche Diagnostics GmbH.

Disposal of all waste should be in accordance with local regulations.

ASSAY PROCEDURE

Preparation of reagent mixture and amplification must be carried out in physically separated areas using dedicated laboratory equipment. See "PROCEDURAL NOTES" for further information.

Run Controls

The product provides negative control, positive control, and internal control (IC) to monitor the reliability of the results for the entire batch of specimens from sample extraction to PCR amplification.

Assay Procedure

Use disposable gloves and dedicated laboratory coats in each area. Change gloves often (e.g. when entering a new working area).

PCR Setup (in first and second Pre-PCR areas). **Note:** use the first pre-PCR area for preparation of PCR reagent mix, the second pre-PCR area for adding the RNA.

1. **Thaw reagents** (first pre-PCR area). Remove **CoV2 Reagent**, **CoV2 Positive Control**, and **CoV2 Negative Control** from the from the freezer, and place them to the PCR preparation area. The Enzyme mix does not freeze at -30 – -16 °C but gelling may occur. Keep the CoV2 Reagent **protected from light**. Thaw the Enzyme mix on ice or at +2–+6 °C until it has returned to liquid state. Allow the other reagents to thaw for 30 minutes to bring them to room temperature (+19–+25 °C) before use. Keep the CoV2 Enzyme Mix in the freezer until needed in step 5 below.

2. **Thaw samples**. Remove **nucleic acid samples** to be analyzed from the freezer and place them to the second pre-PCR area.

3. Vortex-mix the thawed reagents and centrifuge them at low speed for a few seconds to collect the liquid to the bottom of the tubes.

4. Mix the thawed RNA samples gently by inverting the vial 10 times and briefly centrifuge the vial to settle the liquid into the vial bottom.

5. Remove the **CoV2 Enzyme Mix** from the freezer. Mix gently by inverting the vial 10 times (avoid formation of foam and air bubbles) and briefly centrifuge the vial to settle the liquid.

6. Prepare PCR reagent mix **on ice** according to the following table. The positive and negative controls should be included in the number of samples (n(samples)).

Component	Volume
CoV2 Reagent A	1 µL / test x n(samples)
CoV2 Enzyme Mix	5 µL / test x n(samples)
Total	6 µL / test x n(samples)

Note: The Enzyme Mix is a viscous material, exercise caution when pipetting to ensure the correct volume is transferred.

7. Close the vial containing the PCR reagent mix and mix gently by inverting the vial 10 times (avoid formation of foam and air bubbles). **Place the unused kit reagents back to freezer** -30 – -16 °C. Keep the reagent mixture on ice and protected from light and bring it to the sample area.

8. Aliquot 6 µL of the PCR reagent mix into each PCR tube/each well on a PCR plate.

9. Move to second pre-PCR area, and add 14 µL of extracted nucleic acid into each tube or well containing PCR mix, close the tube lids or seal PCR plate tightly using a clear adhesive PCR seal and seal/film applicator. Vortex the tubes carefully and centrifuge them briefly to remove any bubbles. Alternatively, centrifuge the plate for 1 min at 1600 x g and at +19–+25 °C. Proceed to step 10 without delay. Transfer the tubes or PCR plate to the PCR area.

Amplification (in PCR area)

10. Place the PCR tubes/PCR plate from step 9 in a real-time PCR cycler.

11. Set thermal cycling conditions as following for PCR amplification and fluorescence detection.

Step	Temperature	Time	Number of Cycles
1	+25°C *	2 minutes	1
2	+50°C	15 minutes	1
3	+95°C	2 minutes	1
4	+95°C	3 seconds	45
	+60°C **	30 seconds	

* If the temperature cannot be set to 25°C in the cycler (e.g. LightCycler® 480), keep the PCR plate at room temperature for two minutes before starting the amplification run.
** Detect fluorescence signal during the final +60 °C step.

Set fluorescence channels as below:

Analyte	Internal Control (IC)	N-gene	ORF1ab-gene
Detection channel	Cy5®	FAM™	VIC™ or HEX™

PROCEDURAL NOTES

1. Laboratories should have three physically separated working areas [3]: the first pre-PCR area for preparation of PCR reagent mix, the second pre-PCR area for adding the RNA, and the post-PCR area for amplification and product detection. If three separate laboratory rooms are not available, hoods or dead air boxes with ultraviolet light attachments can provide a clean pre-PCR area and the hoods or dead air boxes can be placed in the same room with pre-PCR area for RNA addition. The area used for amplification and product detection should be separate from pre-PCR area(s).

2. To prevent contamination, laboratories should implement a workflow that minimizes the movement from "dirty" to "clean" areas during PCR work. Clean refers to pre-PCR areas without amplification product and where the amplification reactions are set up. Dirty area refers to post-PCR laboratory areas with amplification products, where amplification occurs, and where post-PCR detection methods are used. The workflow from "clean" to "dirty" should be considered also during regular cleaning of the laboratory areas.

3. Cleaning of working surfaces of the pipetting areas regularly with diluted bleach, rinsing with sterile water and drying is an effective way to prevent contamination (use freshly prepared 10% bleach i.e. approximately 0.5% sodium hypochlorite). In addition, use of ultraviolet lights placed over the working surfaces may help to prevent contamination. Protect yourself from ultraviolet light according to instructions of the instrument manufacturer.

4. Each working area should have dedicated pipettors, plastic ware, marker pens, and glassware. Aerosol resistant barrier pipette tips should be used for pipetting of the reagents and setting up the amplification reactions.

5. Close the reagent vials immediately after use to avoid unnecessary exposure to possible sources of contamination.

6. Store the seals in their original package and avoid unnecessary exposure to light before use.

7. Store the PCR plates in their original package before use. Avoid unnecessary exposure to possible sources of contamination.

8. Before and after thermal incubation, it is recommended to visually ensure that the plate has been properly sealed. Detached sealing may affect the results by causing evaporation and cross-contamination of the wells.

9. A thorough understanding of this package insert and the Real-time PCR instrument manuals is necessary for successful use of the 3501-0010 SARS-CoV-2 RT-qPCR

Reagent kit. The reagents supplied with this kit are intended for use as an integral unit. Do not use kit reagents after the expiry date printed on the kit label.

10. The recommended speed of the centrifugation is 1600 x *g*. Please note that rounds per minute (rpm) may differ from *g* values depending on the rotor used.

11. Any deviation from the assay procedure may affect the results.

12. Laboratories should follow local regulations and proper practices for disposal of amplification reactions containing the sample material to avoid contamination. Patient sample disposal should comply with local regulations and institutional policies. **Note: after completing the assay procedure, the sealed reaction plates should be discarded without opening them**. The sealed amplification reactions may also be sent to an off-laboratory site for disposal or be discarded in sealed autoclavable plastic waste bags among the other laboratory waste.

CALCULATION OF RESULTS

After the run completion, save and analyze the data according to the instrument instructions. Most instruments will automatically set up the background/baseline parameters and threshold level which will often produce acceptable results. In some cases, the background/baseline and threshold must be set manually for optimal results. The background/baseline usually starts from 3-5 cycles and ends a few cycles before any significant fluorescence amplification occurs. Threshold level should be set to the beginning of the exponential phase of amplification curves and above the background signal such as the signal from the negative control.

Perform data analysis and interpret the results based on the tables listed in the sections "Quality Control" and "Examination and Interpretation of Patient Specimen Results".

Quality Control

Test results from Positive Control and Negative Control should be examined prior to interpretation of specimen results. Positive control and negative control should meet the requirements listed in the below table to ensure valid results. If the controls are not valid, the specimen results cannot be interpreted.

Control	Ct		
	N-gene (FAM™)	**ORF1ab-gene (Cy5®)**	**Internal control (HEX™/VIC™)**
Negative	Undetermined or Ct>40	Undetermined or Ct>40	Undetermined or Ct>40
Positive	Ct≤32	Ct≤35	No requirement

Negative Control: *ORF1ab* and *N* of SARS-CoV-2 or internal control **should not be** detected or Ct should be >40.

Positive Control: both *ORF1ab* and *N* of SARS-CoV-2 should be detected and their Ct values should be ≤35 ≤32, respectively, the Ct value of internal control has no Ct requirement for the positive control.

Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and confirmed to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

The table below lists an example of the expected results for the kit with valid positive control and negative control. Ct cut-off targets presented below are derived from product verification and validation studies, and user should determine own Ct cut-off values for optimal performance.

Ct		Result interpretation
IC (Cy5®)	N(FAM™), ORF1ab (HEX™)	
≤40	Both targets undetermined or >42	SARS-CoV-2 **not detected**
No requirements on the Ct value	Both targets ≤ 42	SARS-CoV-2 **detected**
No requirements on the Ct value	One of the targets ≤ 42	SARS-CoV-2 **detected***
>40 or undetermined	Both targets undetermined or >42	Invalid result, specimen needs to be **re-tested** from re-extraction or re-collected from patient for test.

* In the case of one SARS-CoV-2 target positive/one SARS-CoV-2 target negative, result is suggestive of a sample at concentrations near or below the limit of detection of the test. Specimen can be re-tested from re-extraction.

LIMITATIONS OF THE PROCEDURE

This kit is used for qualitative detection of SARS-CoV-2 RNA from RNA extracted from a human oropharyngeal swab and nasopharyngeal swab sample. The results do not directly reflect the viral load in the original specimens.

This kit is only applicable to specimen types described in the section "INTENDED USE". Testing other types of specimen may cause inaccurate results. The specimens to be tested shall be collected, processed, stored and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.

The PerkinElmer Nucleic Acid Extraction Kit (CMG-1033-S) and chemagic™ 360 instrument are recommended for nucleic acid extraction. If other nucleic acid extraction reagents or equipment are used, they must be verified before use.

Roche Life Science LightCycler® 480 System, Thermo Fisher QuantStudio™, and Bio-Rad CFX 96 Touch are recommended for nucleic acid amplification. Other nucleic acid amplification instruments should be verified before use.

The limit of detection (LoD) is determined based on a 95% confidence of detection. When SARS-CoV-2 presents at or above the LoD concentration in the test specimen, there is a low probability that SARS-CoV-2 is not detected. When SARS-CoV-2 presents below the LoD concentration in the test specimen, there is also a low probability that SARS-CoV-2 can be detected.

When determining LoD of this kit, a known number of SARS-CoV-2 RNA copies were used. The results are only applicable to this kit, and the copy numbers defined by other methods are not necessarily equivalent.

Primers and probes for this kit target highly conserved regions within the genome of SARS-CoV-2. Mutations occurring in these highly conserved regions, although rare, may result in RNA being undetectable.

This kit uses an UNG/dUTP PCR products carryover prevention system that can be effective in preventing contamination caused by PCR products. However, in the actual operation process, only by strictly following the instructions of PCR laboratories can PCR contamination be avoided.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.

The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics or immunosuppressant drugs on assay performance have not been evaluated.

Laboratories are required to report all positive results to the appropriate public health authorities.

ANALYTICAL PERFORMANCE CHARACTERISTICS

Precision[1]

Two samples with RNA targets at 1× LoD and 5× LoD and positive control were tested with 40 replicated in 10 runs using two different instruments and 2 operators. The coefficient of variation (CV%) of the Ct values for these samples was ≤6%.

Analytical Limits[2]

Limit of Detection (LoD) of this kit was determined to be 1 copy/µL or 20 copies/reaction volume for each viral target (N & ORF1ab).

[1] Study performed at Wallac Oy, Turku, Finland.
[2] As above

Analytical Specificity - Cross-reactivity[3]

Human coronavirus (229E, OC43), SARS coronavirus (plasmid), MERS coronavirus (plasmid), adenovirus (type 2, 3, 31, 37 and 51), enterovirus (type A and D), rhinovirus (type A and B), influenza A virus (H1N1, H1N1-2009, H3N2), influenza B virus, respiratory syncytial virus, parainfluenza virus, measles virus, Mumps virus, human cytomegalovirus, Chlamydia pneumoniae, Mycoplasma pneumoniae, Hemophilus influenzae, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus saliva, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Epstein-Barr virus, herpes simplex virus type I, herpes simplex virus type II, human immunodeficiency virus type I (HIV-1), human immunodeficiency virus type II (HIV-2) and human genomic DNA have been tested with another kit using the same target sequences. The results were all negative for SARS-CoV-2, no cross-reactivity for used primer and probe sequences have been found with these pathogens or DNA.

WARRANTY

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by the manufacturer may affect the results, in which event Wallac Oy and its affiliates disclaim all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

Wallac Oy, its affiliates and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

REFERENCES

[1] Laboratory testing for 2019 novel coronavirus (SARS-CoV-2) in suspected human cases, World Health Organization, 2020.

[2] China CDC Virus Disease Control and Prevention. Novel coronavirus nucleic acid detection primer and probe sequences (Specific Primers and Probes for Detection Novel coronavirus 2019) [EB / OL]., 2020-01-21.

[3] Health Protection Agency. Good Laboratory Practice when Performing Molecular Amplification Assays. National Standard Method QSOP. 2018; Q 4, Issue no: 5.

April 2020

[3] Study performed at Suzhou SYM-BIO LifeScience Co., Ltd.