DIAGNOVITAL

SARS-CoV-2 Real-Time PCR Kit

Qualitative RT-PCR-based detection of SARS-CoV-2

For *in vitro* diagnostic use. For professional use only

CE IVD

REF Σ

09065025 25 tests

09065050 50 tests

09065100 100 tests

Table of Contents

Intended Use

This document describes the use of real-time RT-PCR assays for the in vitro qualitative detection of 2019-Novel Coronavirus (SARS-CoV-2) in respiratory specimens. The SARS-CoV-2 primer and probe sets are designed for the specific detection of SARS-CoV-2.

DIAGNOVITAL® SARS-CoV-2 Real-Time PCR Kit is an *in vitro* nucleic acid amplification assay for qualitative detection of 2019-Novel Coronavirus (SARS-CoV-2) in respiratory specimens using RTA Viral Nucleic Acid Isolation Kit and BIO-RAD CFX96-IVD or Rotor-Gene 3000/6000 or Applied Biosystems 7500 or Quant Studio 5 Real-Time PCR Detection Systems for amplification, detection and analysis.

The kits follow CDC's and WHO's latest detection guidelines.

Product Description

DIAGNOVITAL® SARS-CoV-2 is a real-time RT-PCR-based detection system for the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is considered a novel human coronavirus that is genetically distinct from the common human coronaviruses (229E, NL63, OC43, HKU1), which cause seasonal acute respiratory illness. It is also genetically distinct from the two newer human coronaviruses, MERS-CoV and SARS-CoV.

DIAGNOVITAL® SARS-CoV-2 detects the presence of 2 different and highly specific gene sequences of SARS-CoV-2: E gene and RdRP gene. All 2 assays must be tested positive to confirm the sample as SARS-CoV-2-positive.

Additionally, a non-infectious positive control and a negative human extraction control are included. Human Extraction Control (HEC) is needed to ensure appropriate RNA extraction, purification and reverse transcription and all reagents involved in reaction. The Human Extraction Control (HEC) master mix contains primers and probe for an endogenous human target, which is extracted from the swab during the extraction step. We don't put an external DNA or RNA template as extraction control, since we already get human target during extraction. The positive control is used to confirm functionality of the assays and overall PCR performance, the negative human extraction control is included to evaluate the quality of the RNA isolation independently from the SARS-CoV-2 assays.

QPCR-BASED DETECTION OF SARS-CoV-2

The first step in the detection of SARS-CoV-2 is the conversion of viral RNA into cDNA. Afterwards, the target sequences unique for SARS-CoV-2 are specifically amplified with amplification monitored in real time through the use of fluorescently labelled probes: upon incorporation into the newly amplified DNA strands, the fluorophore (FAM™) is released and an increase in fluorescence signal can be observed.

Due to the intrinsic mutation rate of coronaviruses, it is possible that mutations in the target sequence occur and accumulate over time. This can lead to false-negative results with a PCR-based detection approach. **DIAGNOVITAL® SARS-CoV-2** addresses this issue by using 3 detection assays on 3 different target sequences to minimize the chance of false-negative results caused by an altered target sequence.

If samples are tested negative in one or more assays, additional complementary testing may be required. The original target sequences for SARS-CoV-2 are included as a non-infectious target positive control (TPC) to check the integrity of the detection assays.

Samples tested positive should always be confirmed through complementary methods and additional analysis in an independent laboratory.

Materials Provided

	Reagents	Quantity and Volume (25 tests)	Quantity and Volume (50 tests)	Quantity and Volume (100 tests)
1	50X VitaScript™ Reverse Transcriptase	1 × 27,5 µl	1 × 165 µl	1 × 330 µl
2	Diagnovital® 2X qPCR Mastermix E	1 × 420 µl	1 × 825 µl	1 × 1650 µl
3	Diagnovital® 2X qPCR Mastermix RdRP	1 × 420 µl	1 × 825 µl	1 × 1650 µl
4	Diagnovital® 2X qPCR Mastermix HEC	1 × 420 µl	1 × 825 µl	1 × 1650 µl
5	SARS-CoV-2 Target Positive Control (TPC)	1 × 45 µl	1 × 75 µl	1 × 150 µl
6	Nuclease-free dH_2O	1 × 1000 µl	1 × 1000 µl	1 × 1000 µl

Additional Materials Required

- Suitable means & equipment for nucleic acid extraction
- Real-time PCR detection system equipped for FAM™ detection
- Adjustable pipettes & fitting filtered pipette tips
- Appropriate personal protective equipment & workspaces for working with potentially infectious samples
- Surface decontaminants such as DNAZap™ (Life Technologies), DNA Away™ (Fisher Scientific), RNAse Away™ (Fisher Scientific), 10% bleach (1:10 dilution of commercial 5.25-6.0% sodium hypochlorite)
- Nuclease-free tubes / strips / plates to prepare dilutions, mastermixes etc.
- Nuclease-free tubes / strips / plates corresponding to the PCR device
- Suitable storage options for reagents and specimen (4°C, -20°C, -70°C)

Storage

- Store all components at -20°C and avoid repeated freeze and thaw cycles.
- Protect the 2X qPCR mastermixes from light as prolonged exposure can diminish the performance of the fluorophores.
- If the kit components have been damaged during transport, contact A1 Life Sciences. Do not use as performance may be compromised.
- Keep reagents separate from sample material to avoid contamination.
- Do not use after the designated expiry date.

Performance Characteristics

Analytical sensitivity

Analytical sensitivity was analyzed by use of a dilution series of DIAGNOVITAL SARS-CoV-2 Reference samples. A dilution series of a DIAGNOVITAL SARS-CoV-2 Reference samples was prepared to give the final concentrations of 300, 100, 30 and 10 copies/ml. Each dilution was tested in 24 replicates. Lower limit was calculated by probit analysis done by PASW Statistics 18 program. For each genotype/subtype, Limit of Detection (LoD) values and 95 % confidence ranges are summarized in Table 1.

Table 1: SARS-CoV-2qPCR Kit - Limit of Detection (LoD) values and 95 % confidence ranges

Target gene	Limit of Detection (copies/ml)	95 % confidence lower limit	95 % confidence upper limit
E	38	33	50
RdRP	38	31	58

Precision

In this study, precision of the kit was evaluated for intra-assay, inter-assay, inter-batch, by using RTA Viral NA Isolation Kit (Cat No: 09029) and different specimen types (oropharyngeal vs. nasopharyngal swabs). For each target gene and different assay, 24 replicates of 10^3 copies/ml DIAGNOVITAL SARS-CoV-2 Reference samples were used. Descriptive statistics were analyzed by IBM SPSS Statistics program. Overall precision assays associated with Ct values were summarized in Table 2.

Table 2: Overall descriptive statistics of SARS-CoV-2 precision data.

Descriptive Statistics					
	N	**Mean**	**Std. Deviation**	**Variance**	**Coefficient of variation (%)**
Target gene E	**96**	**23,6175**	**0,139198**	**0,019269875**	**0,595032**
Target gene RdRP	**96**	**24,6675**	**0,084034**	**0,007553**	**0,340342**

Diagnostic specificity

SARS-CoV-2 RNA negative clinical specimens were analyzed to determine the diagnostic specificity of DIAGNOVITAL SARS-CoV-2Real Time PCR Kit. 30 SARS-CoV-2 RNA negative clinical oropharygal swab specimens and 40 SARS-CoV-2 RNA negative clinical oropharygal swab specimens and 30 bronchoalveolar lavage specimens were used. None of the 100 SARS-CoV-2 negative clinical specimens gave positive test result for SARS-CoV-2. Diagnostic specificity of DIAGNOVITAL SARS-CoV-2 Real Time PCR Kit is 100 %. All of the Human Extraxtion Controls (HEC) of tests gave positive result.

Cross-reactivity

To examine the specificity of an assay, cross-reactivity studies should be performed for potential cross-reactive markers. In this study, the specificity of the assay was evaluated by testing. 9 reference organism and 11 clinical specimens which were positive.

DIAGNOVITAL SARS-CoV-2 Real Time PCR Kit do not show any cross-reactivity with other potential cross-reactive markers given in the table 3 below:

Table 3: Potential cross-reactive markers tested in the study.

Organism	*Source*
Human Adenovirus	NIBSC (Cat. No: 16/324)
Parainfluenza virüs	ATCC VR-93
Influenza A	ATCC VR-95
Influenza A H5N1	ATCC VR-1609

Organism	*Source*
Influenza A H1N1	ATCC VR-1672
Influenza A H3N2	ATCC VR-822
Influenza A H7N7	ATCC VR-1641
Influenza B	ATCC VR-101
Parainfluenza 1	ATCC VR-94
Parainfluenza 2	ATCC VR-92
Parainfluenza 3	ATCC VR-93
Parainfluenza 4	ATCC VR-579
Human Metapneumovirus (hMPV)	ATCC VR-3250SD
Human Enterovirus V71	ATCC VR-1432
Human respiratory syncytial virus	ATCC VR-154
Human Coronavirus NL63	ATCC VR-3263SD
Human Coronavirus HKU1	ATCC VR-3262SD
Human Coronavirus 229E	ATCC VR-740
Betacoronavirus 1 OC43	ATCC VR-1558D
MERS Coronavirus	ATCC VR-3248SD

Considerations Before Starting

BIOSAFETY

- Wear appropriate personal protective equipment (e.g. gowns, powder-free gloves, eye protection) when working with clinical specimen.
- Specimen processing should be performed in a certified class II biological safety cabinet following biosafety level 2 or higher guidelines.
- For more information, refer to:
  - Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (SARS-CoV-2) https://www.cdc.gov/coronavirus/SARS-CoV-2/guidelines-clinical-specimens.html
  - Biosafety in Microbiological and Biomedical Laboratories 5th edition available at http://www.cdc.gov/biosafety/publications/.
- The use of **DIAGNOVITAL® SARS-CoV-2** is restricted to trained laboratory personnel only.

SPECIMENS

Only use appropriate specimens for testing, such as:

- Respiratory specimens including nasopharyngeal / oropharyngeal aspirates or washes, nasopharyngeal / oropharyngeal swabs, broncheoalveolar lavage, tracheal aspirates and sputum.
- Swab specimens should be collected only on swabs with a synthetic tip (such as polyester or Dacron®) with plastic shafts. Swabs with calcium alginate or cotton tips with wooden shafts are not acceptable

SPECIMENS - HANDLING AND STORAGE

- Specimens can be stored at 4°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70°C or lower.
- Extracted nucleic acids should be stored at -70°C or lower.

Do not use specimens if

- they were not kept at 2-4°C (≤ 4 days) or frozen at -70°C or below.

- they are insufficiently labelled or lack documentation.
- they are not suitable for this purpose (see above for suitable sample material).
- the specimen volume is insufficient.

Sample Preparation

- The performance of RT-PCR assays strongly depends on the amount and quality of sample template RNA. It is strongly recommended to qualify and validate RNA extraction procedures for recovery and purity before testing specimens.
- Suitable nucleic acid extraction systems successfully used in combination with **DIAGNOVITAL DETECTION KITS** include: RTA Viral NA Isolation Kit from Swabs, bioMérieux NucliSens® systems, QIAamp® Viral RNA Mini Kit, QIAamp® MinElute Virus Spin Kit or RNeasy® Mini Kit (QIAGEN), EZ1 DSP Virus Kit (QIAGEN), Roche MagNA Pure Compact RNA Isolation Kit, Roche MagNA Pure Compact Nucleic Acid Isolation Kit, and Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit, and Invitrogen ChargeSwitch® Total RNA Cell Kit.
- Store and keep residual specimens and extracted nucleic acids at -70°C.
- Only thaw the number of specimen extracts that will be tested in a single day.
- Do not freeze/thaw extracts more than once before testing as each freeze/thaw cycle will decrease the RNA quality.
- It may be possible to use patient samples directly, depending on the sample type. However, this may require a prior lysis step and titration of the amount on sample that can be used without inhibiting the reaction. This procedure has not been validated, use of isolated RNA is recommended.

Reaction Setup

1. Make sure that all necessary equipment and devices are suitable, calibrated and functional before starting the experiments.

2. Decontaminate equipment and workspace and prepare everything needed for the following experiment to keep the workflow short and repeatable.

3. Switch on the PCR detection system and program it to avoid delays after setting up the reactions.

4. Thaw all components of **DIAGNOVITAL® SARS-CoV-2** on ice and mix gently but thoroughly to ensure even distribution of components. Collect liquid at the bottom of the tube with a quick spin (via microcentrifuge).

5. For first tests, prepare a dilution series of your RNA to determine your ideal concentration window. The recommended sample volume is 4 µl / reaction.

6. Set up your **Mastermix Plate**:

   a. Always prepare control reactions with nuclease-free dH_2O instead of sample material (**NTC**) to detect contamination in your reagents.

   b. Always include the assay for the negative human extraction control (**HEC**) to evaluate the quality of your RNA isolate.

   c. When using the provided target positive control (**TPC**), use **4 µl / reaction** and add nuclease-free dH_2O to 20 µl (max volume of the reaction should be 20µ).

   d. > 2 replicates / sample are strongly recommended.

   e. Prepare enough mastermix for all planned reactions. It is recommended to prepare mastermix for 2 additional reactions to compensate for pipetting inaccuracies.

Component	**Volume**
50X VitaScript™ Reverse Transcriptase	1 µl
Diagnovital® 2X qPCR Mastermix (E / RdRP / HEC)	15 µl
isolated sample RNA / TPC	4 µl

f. Distribute the mastermix to your strips/plate. An example setup is given in **Fig 1**).

	1	2	3	4	5	6	7	8	9	10	11	12
A	E	E	E	E	E	E	E	E	E	E	E	E
B	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP	RdRP
C	HEC	HEC	HEC	HEC	HEC	HEC	HEC	HEC	HEC	HEC	HEC	HEC
D												
E												
F												
G												
H												

Figure 1: Example pipetting scheme for the distribution of mastermixes with the individual assay mixes

7. Transfer the Mastermix Plate to a separate workspace to add the sample material. Preparing reagents and final reaction setup in separate workspaces helps to avoid contamination of equipment and reagents with sample material.

   a. Prepare negative reactions first and seal them before handling positive samples. It is recommended to only bring potentially positive sample material and the included target positive control to the workspace once the NTC is prepared and sealed.

   b. Add your samples to the Mastermix Plate. An example setup is given in **Fig 2**).

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	TPC
B	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	TPC
C	NTC	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	TPC
D												
E												
F												
G												
H												

Figure 2: Example pipetting scheme for the addition of samples. The bottom half of the plate could be used for replicates with an identical setup

   c. Keep reactions on ice until transferring them to the PCR device.

8. Transfer the reactions to the PCR device, then proceed according to these guidelines:

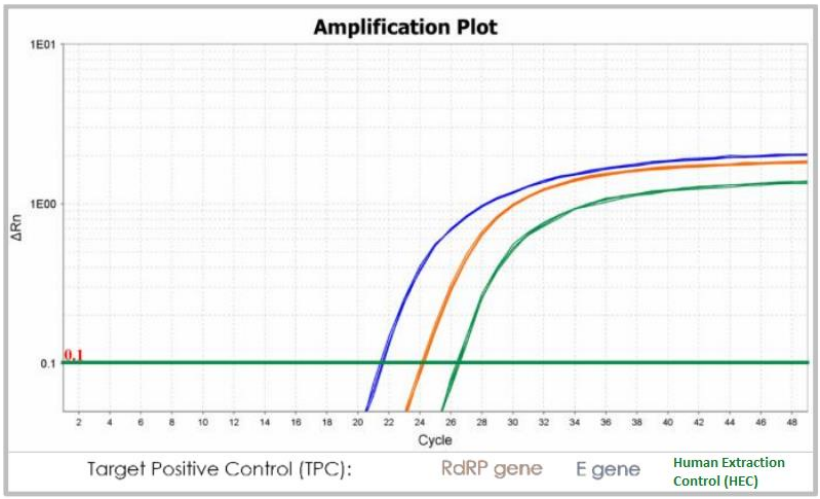
Step	Cycles	Temperature	Duration
Reverse Transcription	1	45°C	20 minutes
Initial Denaturation	1	95°C	10 minutes
Amplification	45	95°C	15 seconds
		58°C*	45 seconds

*Enable Data Collection for **FAM™**.

9. Once the run is finished, do not open the reaction tubes to avoid contamination and discard according to local guidelines and regulations. Do not autoclave as this may contaminate laboratory equipment with amplicons.

Analysis & Troubleshooting

EXEMPLARY RESULT



- **dH_2O controls (NTC) must not give a positive Ct for any assay**. If they do, the reaction was contaminated with sample RNA / DNA. Decontaminate equipment and workspace and repeat the reactions.
- **For a sample to be considered positive for SARS-CoV-2, all 2 assays (E / RdRP) must give positive Ct values**. If the **HEC** fails to amplify, the sample must still be considered positive.
- **For a sample to be considered negative for SARS-CoV-2, none of the 2 assays (E / RdRP) must give positive Ct values**. The **HEC** must give a positive Ct value (< 35 cycles) for these samples to ensure that sample material of suitable quality was present.
- **All reactions containing RNA isolate must give positive Ct values for the HEC assay. The Ct values should be < 35 cycles**. Failure to amplify the negative human extraction control indicates a flawed RNA extraction or loss of RNA isolate due to RNAse contamination. The sample is not sufficient, results cannot be interpreted.

- **When using the TPC for SARS-CoV-2, a positive Ct for all 2 assays must be observed. The Ct value for the TPC should be < 35 cycles**. If the Ct value does not correspond to the expected value or not all assays are tested positive, PCR was compromised. Check the reaction setup and PCR device settings and repeat the reactions. Repeated freeze and thaw cycles of the TPC can compromise its quality resulting in late Ct values.

- **If no amplification signal is observed for any assay, PCR was inhibited**. Check reaction setup and device settings and repeat the RNA extraction if necessary. Results are invalid and cannot be interpreted.

- **If < 3 of the target assays are positive (e.g. only E gene, only RdRP gene etc.), results are inconclusive**. Check reaction setup and device settings and repeat the RNA extraction if necessary. Results are invalid and cannot be interpreted.

E	RDRP	HEC	Interpretation
+	+	+	All 2 target sequences for SARS-CoV-2 and the HEC were amplified. The sample is considered positive for SARS-CoV-2.
/	+	+	SARS-CoV-2 target sequence is detected and sample is considered as SARS-CoV-2 positive. A positive SARS-CoV-2 and a negative Sarbecovirus result is suggestive for low viral NA concentration or mutation in Sarbeco sequence.
/	/	+	Only the target sequence for the HEC was amplified. The sample is considered negative for SARS-CoV-2.
+	/	+	Sample is presumptive positive for SARS-CoV-2. This result suggests low viral RNA, a mutation in SARS-CoV-2 sequence or may indicate other Sarbecovirus (SARS or SARS-related Coronavirus) infection.
/	/	/	PCR was inhibited, results are invalid.
+	+	/	Expected result for TPC

Limitations

- For reliable results, it is essential to adhere to the guidelines given in this manual. Changes in reaction setup or cycling protocol may lead to failed experiments.
- Depending on the sample matrix, inhibitors may be present in the isolated RNA and disable reverse transcription and / or PCR amplification. If this is the case, another sample type or isolation method may be beneficial.
- Spontaneous mutations within the target sequence may result in failure to detect the target sequence.
- Results must always be interpreted in consideration of all other data gathered from a sample. Interpretation must be performed by personnel trained and experienced with this kind of experiment.

RTA LABORATUVARLARI BİYOLOJİK ÜRÜNLER İLAÇ VE MAKİNE SAN. TİC. A.Ş.
Geposb Cumhuriyet Cad. No:3
41400 Gebze / Kocaeli / Turkey

Tel: +90 262 648 5300
E-mail: rta@rtalabs.com.tr
Web: www.rtalabs.com.tr