DIAGNOVITAL

DIAGNO4PLEX NS SARS-CoV-2 Real-Time PCR Kit

Qualitative RT-PCR-based detection of SARS-CoV-2 and N501Y Variants

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

IVD CE

REF Σ

09075025 25 tests

09075050 50 tests

09075100 100 tests

Table of Contents

Intended Use 3
Product Description 3
Materials Provided 4
Additional Materials Required 4
Storage 4
Performance Characteristics 4
Considerations Before Starting 6
Sample Preparation 6
Reaction Setup 7
Analysis & Troubleshooting 8
Limitations 11
Trademarks 11

Intended Use

DIAGNO4PLEX NS SARS-CoV-2 Real Time PCR Test is a real-time RT-PCR-based diagnostic test for the in vitro qualitative detection and discrimination of wildtype SARS-CoV-2 and N501Y variants in respiratory specimens.

DIAGNO4PLEX NS SARS-CoV-2 Real Time PCR Test detects wildtype SARS-CoV-2 RNA and N501Y variant RNA in nasopharyngeal and oropharyngeal swab samples during infection. Positive results indicate the presence of SARS-CoV-2 RNA and allow discrimination between N501 and Y501 in the spike protein. Spike protein of wildtype SARS-CoV-2 has asparagine(N) amino acid at 501th location define as N501. SARS-CoV-2 variant has tyrosine(Y) amino acid at 501th location in Spike protein define as Y501. This amino acid change in the spike protein causes the strong binding of SARS-CoV-2 to ACE. Increasing trend of the VOC-202012/01 prevalence is the indicator of its higher transmission rates. The 501Y.V2 and P.1 variants do not seem to have a higher transmission rate, but they are also alarming due to their potential of causing more severe illness or escaping from the neutralizing antibodies. In Positive results; clinical correlation with patient history and other diagnostic information must be considered to determine the actual patient infection status. Positive results do not exclude bacterial infection or co-infection with other viruses.

Negative results do not exclude an infection with SARS-CoV-2 and must 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 use of **DIAGNO4PLEX NS SARS-CoV-2** Real Time PCR Test is intended for use by clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

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

Product Description

DIAGNO4PLEX NS SARS-CoV-2 Real Time PCR Test is a real-time RT-PCR-based detection and discrimination system for wildtype SARS-CoV-2 and N501Y variants.

DIAGNO4PLEX NS SARS-CoV-2 Real Time PCR Test detects the presence of highly specific gene N gene sequence of SARS-CoV-2, sequence specific for human RNA serving as a human extraction control (HEC) and detects wildtype 501N SNP and Y501 SNP in two different channels.

REAL TIME PCR-BASED DETECTION OF SARS-CoV-2

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

With **DIAGNO4PLEX NS SARS-CoV-2** Real Time PCR Test discrimination between the viral targets is achieved through the use of three different fluorophores that are detected in three different channels: FAM™ for wildtype SARS-CoV-2 501N, HEX/VIC for Y501 variants, ROX for SARS-CoV-2 in general and the HEC is detected in the Cy5 channel.

Due to the intrinsic mutation rate of viruses, 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.
Samples tested positive for any of the viruses should always be confirmed through complementary methods and additional analysis in an independent laboratory.

DIAGNO4PLEX NS SARS-CoV-2 Real Time PCR Test is compatible with every qPCR cycler with calibrated FAM™, HEX/VIC, ROX and CY5 channel.

Materials Provided

	Reagents	Quantity and Volume (25 tests)	Quantity and Volume (50 tests)	Quantity and Volume (100 tests)
1	PCR Master Mix	1 × 375 µl	1 × 750 µl	1 × 1500 µl
2	Positive Control	1 × 38 µl	1 × 75 µl	1 × 150 µl
3	Nuclease-free dH_2O	1 × 38 µl	1 × 75 µl	1 × 150 µl

IMPORTANT! The table above reflects the standard kit color scheme. Due to supplier issues during the COVID-19 crisis, individual tube cap colors may be substituted due to availability. Always check the labeling of the reagent prior to use.

Additional Materials Required

- Suitable means & equipment for nucleic acid extraction
- Real-time PCR detection system equipped for FAM™, HEX/VIC, ROX/TEXAS RED and CY5 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, master mixes 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 -15°C /-25°C and avoid repeated freeze and thaw cycles.
- Protect the qPCR master mix from light as prolonged exposure can diminish the performance of the fluorophores.
- If the kit components have been damaged during transport, contact RTA Laboratories. 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® NS SARS-CoV-2 Reference samples. A dilution series of a DIAGNOVITAL® NS SARS-CoV-2 Reference samples was prepared to give the final concentrations of 3000, 1000, 300 and 100 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: DIAGNO4PLEX NS SARS-CoV-2 PCR 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
N	156	150	160
N501	500	490	560
Y501	500	490	560

Diagnostic specificity

SARS-CoV-2 RNA negative clinical specimens were analyzed to determine the diagnostic specificity of DIAGNO4PLEX NS SARS-CoV-2 Real Time PCR Kit. 30 SARS-CoV-2 RNA negative clinical oropharyngeal swab specimens and 30 SARS-CoV-2 RNA negative clinical nasopharyngeal swab specimens and 30 bronchoalveolar lavage specimens were used. None of the 90 SARS-CoV-2 negative clinical specimens gave positive test result for SARS-CoV-2 variants. Diagnostic specificity of DIAGNOVITAL® NS SARS-CoV-2 Real Time PCR Kit is 100 %. All of the Internal Controls (RNAseP) have been tested positive.

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. 20 reference organisms.

DIAGNO4PLEX NS SARS-CoV-2 Real Time PCR Kit do not show any cross-reactivity with other potential cross-reactive markers given in the table 2 below:

Table 2: Potential cross-reactive markers tested in the study

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

Sample	*Source*
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 specimens.
- 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/2019-nCoV/guidelines-clinical-specimens.html
- Biosafety in Microbiological and Biomedical Laboratories 6th edition available at http://www.cdc.gov/biosafety/publications/.
- The use **of DIAGNO4PLEX NS SARS-CoV-2** Real Time PCR Test and data evaluation is restricted to trained laboratory personnel only.
- Good laboratory practice is essential for optimal performance of this assay. Special care must be taken avoid contamination of the components of the kit. All reagents must be closely monitored for impurities and contamination. Discard suspicious reagents according to local guidelines and regulations.

SPECIMENS

Only use appropriate specimens for testing, such as:

- Respiratory specimens including nasopharyngeal / oropharyngeal swabs and bronchoalveolar lavage.
- 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 2-8°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -20°C or lower.
- Extracted nucleic acids should be stored at -20°C or lower.

Do not use specimens if

- they were not kept at 2-8°C (≤ 4 days) or frozen at -20°C or lower.
- 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* like Generotex96, Ziexpress32 successfully used in combination with **DIAGNOVITAL DETECTION KITS** include: RTA Viral Nucleic Acid Isolation kit, RTA Viral RNA Isolation kit, bioMérieux NucliSens® systems, QIAamp® Viral RNA Mini Kit, QIAamp® MinElute Virus Spin Kit or RNeasy® Mini Kit (QIAGEN), EZ1 DSP Virus Kit (QIAGEN), Roche Mag NA 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, Invitrogen ChargeSwitch® Total RNA Cell Kit.
- *Compatible Real Time PCR systems*: Roche LightCycler 480, Roche LightCycler 96, Qiagen Rotor Gene Q, Qiagen Rotor Gene Series, Corbett Realtime PCR, BMS Mic, Biorad CFX Connect, Biorad CFX96, Biorad CFX Touch, Applied Biosystem ABI 7500, Applied Biosystem ABI 7500 Fast, Applied Biosystem ABI StepOne, Applied Biosystem ABI StepOne Plus, Thermo Scientific Quant Studio 5, Slan Realtime PCR, Tianlog Gentier 96E, Tianlong Gentier 96R, Stratagene Mx3500p, Azure Biosystems™ Cielo™ 3 and 6 real-time PCR Systems.
- Store and keep residual specimens and extracted nucleic acids at -20°C or -80°C.
- Only thaw the number of specimen extracts that will be tested in a single day.
- Do not freeze/thaw extract 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® NS 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. The **PCR Master Mix** provided with **DIAGNO4PLEX NS SARS-CoV-2 Real-Time PCR Kit** is ready to use. One reaction will be prepared for each sample. A separate reaction should be prepared for Negative Control (NTC) and Positive Control (PC).

Component	**Volume (µl)**
PCR Master Mix	15
RNA Isolate/ PC/ NTC	5
Total	**20**

6. Distribute **15 µl** PCR Master Mix to your strips/plate and add **5 µl your samples**. (An example setup is given in **Fig1**).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	PC
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S89
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S90
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S91
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S92
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S93
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	S94
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	NTC

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

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

Step	**Cycles**	**Temperature**	**Duration**
Reverse Transcription	1	50°C	5 minutes
Initial Denaturation	1	95°C	5 minutes
Amplification	40	95°C	5 seconds
		60°C*	45 seconds

*Enable Data Collection for **FAM™** (501N SARS-CoV-2), **HEX/VIC** (N501Y SARS-CoV-2 Variant), **ROX/TEXAS RED** (SARS-CoV-2) and **Cy5** (HEC). Do not set ROX as passive reference since the channel is used for detection.

8. 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

EXEMPLAR RESULTS

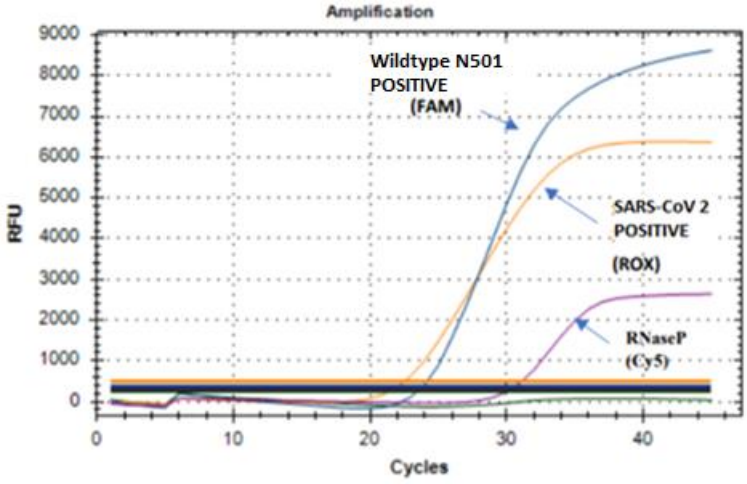

Figure 2: *Blue Curves: N501 Wild Type sample at the FAM channel, Orange Curves: SARS CoV 2 positive sample at the ROX channel, Purple Curves: internal control at the Cy5 channel.*

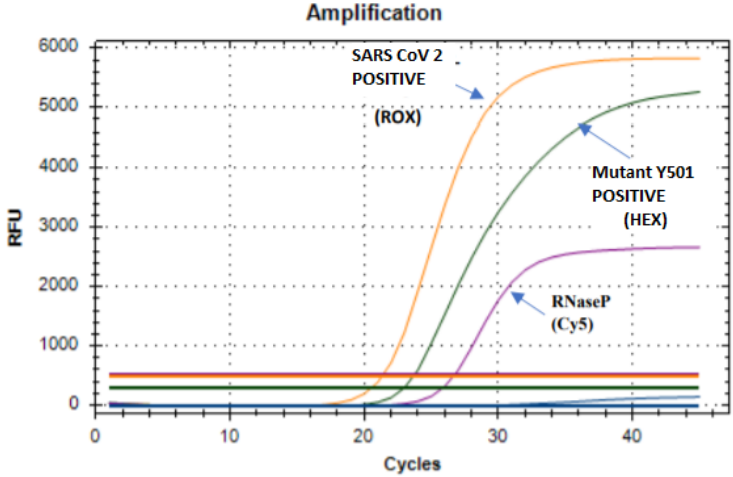

Figure 3: *Green Curves: Y501 Mutant positive sample at the HEX channel, Orange Curves: SARS CoV 2 positive sample at the ROX channel, Purple Curves: internal control at the Cy5 channel.*

- **dH$_2$O 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 Wildtype N501 SARS-CoV-2, the FAM™ channel and the ROX channel must give a positive Ct value**. Amplification of the HEC in Cy5 channel is expected around Ct 20-38. Should the HEC fail to amplify, the sample must still be considered positive. This outcome is possible when having an unusually high virus titer, or the sample was not of human origin, but cell culture derived or analysis of surface contamination.

- **For a sample to be considered positive for mutant Y501 SARS-CoV-2 variant, the HEX/VIC channel and the ROX channel must give a positive Ct value**.

- **For a sample to be considered positive for SARS-CoV-2, the Texas Red/ ROX channel must give a positive Ct value.** This result might appear in cases of a very low viral titer insufficient for the allelic discrimination of N501 and Y501.

- **All reactions containing RNA isolate must give positive Ct values for the internal control assay. The Ct values should be < 38 cycles**. Failure to amplify the internal control indicates a flawed RNA extraction or loss of RNA isolate due to RNase contamination. The sample is not sufficient, results cannot be interpreted.

- **For the positive control, a positive Ct at the FAM, HEX/VIC, ROX/TEXAS RED and CY5 channels must be observed. The Ct value for the positive control should be Ct<35**. If the Ct value does not correspond to the expected value or the **positive control** was not tested positive, PCR was compromised. Check the reaction setup and PCR device settings and repeat the reactions. Repeated freeze and thaw cycles of the **positive control** can compromise its quality resulting in late Ct values.

- If Ct is <38 for each of the FAM, HEX, ROX, CY5 channels, the result in the respective channel should be considered as **POSITIVE**, if Ct is > 38 or no value is received, the result in the relevant channel should be considered as **NEGATIVE**.

FAM	HEX/VIC	Texas Red/ ROX	Cy5	Result
-	-	-	+	The sample does not contain **any SARS-CoV-2 RNA**. The control was amplified successfully. The sample is considered **negative for SARS-CoV-2.**
+	-	+	+	The sample is **positive for wildtype N501 SARS-CoV-2**.
-	+	+	+	The sample is **positive for Y501 variant SARS-CoV-2**.
-	-	+	+	The sample is **positive for SARS-CoV-2**.
-	-	-	-	No amplification in any channel indicates flawed RNA isolation, sample degradation or PCR inhibition. Results cannot be interpreted.
+	+	+	-	Expected result for the ***Positive Control (TPC)***.
-	-	-	-	Expected result for the ***Negative Control (NTC)***.

Baseline Setting

The amplification curve baseline is one of the parameters that can affect PCR results. In case the baseline is incorrectly set, a Ct value can be displayed even if no real amplification occurred. Auto threshold is used with **DIAGNO4PLEX NS SARS-CoV-2** Real-Time PCR Kit for PCR Detection systems. If the increase of a sample in any channel is **less than 10%** of the increase of the highest increasing sample in the same channel, this increase is considered as **NEGATIVE**.

Troubleshooting

PROBLEM	POTENTIAL REASONS	SOLUTION
Negative Result for Internal Control	PCR Master Mix may not have been homogenous.	Pipetting should be performed for PCR Master Mix.
	RNA isolation may not be performed as properly.	The study should be repeated from isolation.
	Isolate may include inhibitor.	Real Time PCR stage should be repeated by diluting the isolate 1/10.
Positive increases of NTC samples were observed	Contamination may have occurred.	Contamination may have occurred from the work area to the consumable items being worked on. It is recommended to dispose of consumables and open new ones and clean the environment first with 10% NaClO solution and then with 70% Alcohol.
Results observed at Low Ct	For positive results having Ct > 38	The study should be repeated. If the same result is obtained, the sample is considered negative.

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.

Trademarks

DIAGNOVITAL® NS SARS-CoV-2 Real Time PCR Test, NucliSens® (bioMérieux), QIAamp®, RNeasy® (QIAGEN), ChargeSwitch® (Invitrogen), ROX™, FAM™ (Life Technologies), DNAZap™, DNA Away™, RNAse Away™

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

Symbols

Expiry Date

Lot/ Batch

Catalog Number

Temperature Limitation

Caution

In Vitro Diagnostic Medical Device

Manufacturer

Consult instructions for use or
consult electronic instructions for use

Contains sufficient for (n) amount tests

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