SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes, IVD

Viral RNA dependent RNA polymerase (RdRp) and Nucleocapsid phosphoprotein (N) genes

REF MD04831, 96 reactions
MD04832, 4 x 96 reactions

For professional* in-vitro *diagnostic use only

CE IVD

EN

Instructions for Use (IFU)

IM-002en

VERSION 13/2021, December 2021

NZYTech genes & enzymes
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1. Introduction

In December 2019 a novel acute respiratory disease, termed Coronavirus Disease 2019 (COVID-19), was reported in China and rapidly spread worldwide. The causative agent was identified as Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2). The virus (previously named 2019-nCoV), like the closely related SARS coronavirus (SARS-CoV), belongs to the genus *Betacoronavirus* within the family of coronaviruses. Coronaviruses are enveloped, positive, single-stranded large RNA viruses that infect humans, but also a wide range of animals. SARS-CoV-2 is thought to be of zoonotic origin and likely to have spread from large seafood and animal markets by human-animal contact in the city of Wuhan. The novel coronavirus is highly contagious and is primarily transmitted via respiratory droplets (coughing and sneezing). Early detection of SARS-CoV-2 is vital in providing rapid treatment to infected patients and, thus, to reduce the spread of infections. The most common clinical manifestations of COVID-19 include fatigue, fever and lower respiratory symptoms, such as dry cough and dyspnea. Loss of smell and taste can also occur. In the most critical situations, the infection progresses to severe pneumonia with life-threatening complications such as acute respiratory disease syndrome, organ dysfunction and death. Based on current knowledge, a significant proportion of infections are mild or asymptomatic. A percentage of the population is more vulnerable to the severe form of disease, including older adults (60 years and older), smokers and people with chronic diseases such as heart or lung disease, cancer, diabetes and patients with a weakened immune system. Currently, there is no specific treatment or vaccine available against SARS-CoV-2 infection.

2. Intended Use

NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD) is a molecular test intended for the rapid qualitative detection of Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) nucleic acids in nasopharyngeal or oropharyngeal swabs samples collected from patients. A positive result indicates the presence of SARS-CoV-2 RNA but clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. This kit is intended for use by laboratory trained personnel, specifically instructed in real-time PCR techniques and *in vitro* diagnostics.

3. Principles of the Assay

NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD) provides the complete set of reagents and probes to qualitatively detect the SARS-CoV-2 genome, through common real-time PCR platforms (see required instrument specifications in **Section 6**). The virus RNA dependent RNA polymerase (RdRp) and the Nucleocapsid phosphoprotein (N) genes have previously been identified as highly specific markers for SARS-CoV-2. This NZYTech kit targets specific regions in the RdRp and N genes of SARS-CoV-2 genome to provide the highest sensitivity of detection. SARS-CoV-2 kit primers and probes have 100% homology with >95% of the >5M genome sequences available on the GISAID database, as of November 2021, including complete identity to the Delta (B.1.617.2) and Omicron (B.1.1.529) variants. In addition, primers and probes targeting SARS-CoV-2 display no significant homology with unrelated genomes rendering this test highly specific as there is no cross reactivity with nucleic acids

from other respiratory viral and bacterial organisms. An internal control is included to confirm efficient RNA extraction from human biological samples, as well as the absence of PCR inhibitors, among others. In addition, the test uses external controls (positive low titer control provided with the kit and negative control), as described below. The positive control consists of nucleic acid fragments containing the three target sequences detected by the kit (SARS-CoV-2, RdRp and N genes, and human RP gene). The natural evolution of SARS-CoV-2 implies that new sequence information will become available after the initial design of this kit, which reflects SARS-CoV-2 adaptation strategies. Thus, NZYTech periodically revisits SARS-CoV-2 genomic targets and, if required, will release new versions of this kit.

The qualitative detection of RNA is based on the one-step real-time RT-PCR technology, as this remains the most sensitive method to perform an accurate detection of SARS-CoV-2. By using NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD), RNA isolated and purified with a CE IVD extraction system is retrotranscribed (RT) to cDNA and subsequently amplified by PCR, in a single reaction, using three highly specific primer/probe sets exploiting the so-called TaqMan® principle. During this process, the probes specifically anneal to two regions of the SARS-CoV-2 genome, namely RdRp (within the Orf1ab polyprotein gene) and N genes, in case the sample was extracted from an infected patient. An additional primers/probe set acts as an endogenous internal control to detect nucleic acids of the human ribonuclease P [RNase P gene (RP)], assessing sample quality. In addition, this internal control demonstrates that no reaction inhibition has occurred by PCR inhibitors potentially present in the clinical/environmental samples. To allow identifying the amplification of the three specific targets in a single reaction, SARS-CoV-2 and human RNase P specific probes are differently labelled, with FAM™ and JOE™ reporter dyes, respectively. Note that this panel contains a duplex assay in the same optical FAM channel to report an additive performance of the two PCR assays for SARS-Cov-2 detection. In addition, they are provided in optimized concentrations to make sure amplification of human mRNA, even when present at very high concentrations, does not limit the efficiency of the SARS-CoV-2 primers/probe sets.

4. Kit Composition

NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD) provides a comprehensive set of reagents sufficient to perform 96 RT-PCR reactions in a single step.

Kit Component		**Volume (per vial)**	**Number of Tubes**	
			MD04831	**MD04832**
SARS-CoV-2 MMix (RdRp & N)	NZYSupreme One-step RT-qPCR Master Mix	1050 µL	1	4
SARS-CoV-2 PPMix (RdRp & N)	SARS-CoV-2(RdRp & N genes)/RP primer/probe Mix (FAM™ and JOE™ labelled)	205 µL	1	4
SARS-CoV-2 Pos (RdRp & N)	SARS-CoV-2(RdRp & N genes)/RP Positive Control (1 x 10^4 copies/µL)	105 µL	1	4
NTC	No-template Control	105 µL	1	4

5. Storage, Stability and Handling Conditions

SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD) is shipped refrigerated. All components should immediately be stored at -30°C to -15°C upon arrival. When in use, the kit components should be returned to the freezer promptly after use to minimise the time at room temperature.

- Minimise the number of freeze-thaw cycles by storing in working aliquots. If appropriate, kit components may be aliquoted into smaller volumes after thawing.
- The SARS-CoV-2(RdRp & N genes)/RP primer/probe Mix should be stored protected from light. Particularly, do not expose the NZYSupreme One-step RT-qPCR Master Mix to direct sun light after combining with primers/probe mix.
- If the package that protects the kit arrived damaged, please contact NZYTech.
- Beware to the expiry date indicated on the packaging. NZYTech does not recommend using the kit after the expiry date. On this date, the kit must be discarded following the disposal instructions in **Section 8.2**.

6. Materials and Instrumentation Required but Not Provided

- Real-time PCR Instrument that detects FAM™ and JOE™/VIC©/HEX™ fluorescent dyes (at emission wavelengths of 520 and 555/554/556 nm, respectively). See in **Section 11** the instrument models for which the kit was validated.
- Equipment and consumables for isolating viral RNA from respiratory specimens.
- RNase/DNase free qPCR plasticware: PCR tubes, strips, caps, 96-well plates, adhesive films.
- Pipettors and filter tips (RNase/DNase free).
- Disposable gloves.
- Vortex and centrifuge.

7. Sample Collection and Preparation

Different factors, such as protocol for sample collection from human respiratory specimen (nasopharyngeal or oropharyngeal swabs), sample transport, storage and processing time, are critical to achieve optimal results. The collected samples should be tested as soon as possible. Samples should be transported and stored at low temperatures in accordance with biosafety regulations. RNA or total nucleic acids extracted following a CE IVD protocol are the starting material for NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD). Please ensure RNA samples are suitable in terms of purity, concentration and nucleic acid integrity. An $A_{260/280}$ ratio of ~2 is generally accepted for pure RNA. Since ethanol is a strong Real-Time PCR inhibitor, it is necessary to completely eliminate it prior to the elution of the nucleic acid during extraction. NZYTech kit integrates an internal RNA extraction control reaction that targets human RNA, which is co-purified with viral RNA. Human RNA is amplified with the RNase P (RP) primers/probe set. This is useful for checking the efficiency of RNA isolation and/or the presence of inhibitors during sample processing.

8. Precautions and Warnings

As in any analytical testing procedure, good laboratory practices are essential. Carefully follow the procedures and guidelines provided in this handbook to ensure that the test is performed correctly. Any deviation from them may result in assay failure or cause erroneous results. Due to high sensitivity of the kit, special care must be taken to keep reagents and PCR amplification mixes free from contamination.

8.1 Safety Information

Before using the kit please consult the Safety Data Sheet (SDS) that is available at NZYTech website (www.nzytech.com). Detection of SARS-CoV-2 virus should be performed only by staff trained in the relevant technical and safety procedures in appropriately equipped laboratories. International and national guidelines on laboratory biosafety should be followed in all circumstances.

8.2 Handling and Procedural Requirements

- Only for professional *in vitro* diagnostic use.
- Do not use this kit after expiration date.
- Do not use the test components, if kit sealing is damaged.
- Do not interchange reagents of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- DNase/RNase free disposable plasticware and pipettes should be used in all procedures.
- Use DNase/RNase free filter tips throughout the protocol to prevent aerosol and liquid contamination.
- Sample preparation, reaction set up and amplification should be performed in different working areas.
- Positive control contains a high copy number of templates; it should be opened and processed away from test samples and kit components to avoid cross-contamination.
- Always use the NTC provided in the kit.
- At the end of each testing, clean work surfaces and equipment with a DNA/RNA remover.
- Handle post-amplification plates with care and dispose them immediately after the end of the testing; plates should always be discarded into a proper biohazard container after use.
- Biological samples must be handled as if they are infectious following proper biosafety precautions.
- Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations.
- All results should be interpreted by a health-care professional in the context of patient medical history and clinical symptoms.
- This test cannot rule out diseases caused by other pathogens.
- A negative result for any PCR test does not conclusively rule out the possibility of infection.
- Follow good laboratory practices, wear protective clothing, permanently wear disposable powder-free gloves, use goggles and mask. Do not eat, drink or smoke in the working area.

9. Testing Procedure

Please read the instructions for use carefully before performing the assay. Beware that all pipetting steps and experimental plate set-up should be performed on ice. After the plate is poured start immediately to the one-step RT-PCR protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artefacts that reduce the sensitivity of detection. Prior to the experiment, start to gently mix the reaction tubes provided, centrifuge for 5 seconds to collect contents at the bottom of the tube and place tubes on ice. **We strongly recommend pipetting the SARS-CoV-2(RdRp & N genes)/RP Positive Control last to avoid cross contaminations.**

9.1 Reaction set-up

1. Prepare a RT-PCR mix enough for the number of SARS-CoV-2/RNase P tests to be performed with a 5% additional volume for pipetting losses. Proceed according to the table below that specify the volumes for 1 and *n* tests (where *n* corresponds to the total number of reactions):

Component	**1 test volume (µL)**	***n* tests (*) volume + 5% (µL)**
SARS-CoV-2 MMix	10	*n* x 10.5
SARS-CoV-2 PPMix	2	*n* x 2.1
Final Volume	**12**	***n* x 12.6**

(*) To calculate the total number of reactions needed for each assay, count the number of samples and add two more for the Negative and Positive controls, respectively.

2. Pipette 12 µL of the RT-PCR mix into individual wells according to your real-time PCR experimental plate set-up.
3. For the negative control, add 8 µL of NTC instead of RNA template into the negative control well. The final volume should be 20 µL.
4. For the biological samples, add 8 µL of each RNA sample into the SARS-CoV-2/RNase P wells, according to your experimental plate set-up. The final volume in each well should be 20 µL.
5. For the positive control, add 8 µL of SARS-CoV-2 Pos (RdRp & N) instead of RNA template into the positive control well. The final volume should be 20 µL.
6. Cover and seal the plate with an appropriate optical adhesive film before proceeding with the RT-PCR and detection steps.
7. Place the reaction plate in the real-time PCR instrument and run the RT-PCR protocol according to the section below.

9.2 Programming the real-time PCR instrument

The table below displays a standard protocol optimized on a few platforms. However, these conditions may be adapted and validated to suit different machine-specific protocols.

Suggested RT-qPCR Run Settings

Cycles	Temperature	Time	Step
1	50 °C	20 min	Reverse Transcription
1	95 °C	2 min	Polymerase activation
40	95 °C	5 s	Denaturation
	60 °C	30 s	Annealing/Extension*

**Depending on the equipment used select the proper detection channel. Collect signals (FAM and JOE/VIC/HEX).*

Fluorescent Dyes & Detection Channels

Targets	Fluorescent dye	Detection Channels
SARS-CoV-2	FAM™	FAM
RNase P	JOE™	JOE, VIC or HEX

NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD) was validated for the following Real Time PCR Systems: Applied Biosystem® 7500 FAST, Applied Biosystem® StepOnePlus, Roche Life Science LightCycler® 480 II, Applied Biosystem® QuantStudio 6 Pro, Qiagen Rotor-Gene Q and Bio-Rad® CFX96™. If other equipment is used, the kit should be validated by the user by using previous characterised samples (both positive and negative).

10. Data Analysis

10.1 Run Validation Criteria

The detection of SARS-CoV-2 RNA is performed by detecting two target genome regions, which are both detected in the same fluorescence channel (FAM™). Data analysis is performed by the software of the instrument. Considering performance differences in different real-time PCR instruments, thresholds for the two fluorescence signals (FAM™ and JOE™) are determined automatically by the software with manual adjustments in case this is required. Before analysing samples results, we recommend to verify if the real-time PCR test is valid. Thus, for each plate, please confirm if the results for Positive and Negative controls performed as expected, according to the following criteria:

Positive control: the amplification curves of FAM™ (SARS-CoV-2, RdRp & N genes) and JOE™ (RP) are positive. Positive control is expected to amplify at a Ct<30, both in the FAM and JOE/VIC/HEX channels. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

Negative control (no template reaction): no amplification is detected. If the negative control has one or two amplification curves (FAM and/or JOE/VIC/HEX channels) with a sigmoidal shape, sample contamination may have occurred. Repeat the test following good RT-PCR practices.

If the controls are according with expected, the test is **valid**. Please proceed with interpretation of results for the tested samples.

If any of the controls do not exhibit the expected performance, the assay was compromised or executed improperly and should be considered **invalid**.
Please, repeat the test
If the problem persists contact the manufacturer

10.2 Test Results Interpretation

SARS-CoV-2 is detected if the FAM™ amplification curve displays a sigmoidal shape with a Ct≤35, regardless of what result is obtained for the RP (JOE™) assay.

SARS-CoV-2 is not detected if FAM™ curve is not positive (Ct>35) while the RP (JOE™) displays a positive sigmoidal curve (Ct<40).

The **test is invalid** if the SARS-CoV-2 and RP assays are both negative. The test should be repeated with nucleic acid re-purified from the sample.

The following table summarises the interpretation of principal results (evaluate the overall shape of the amplification curves; **only sigmoidal amplification curves are indicative of true amplification**).

SARS-CoV-2 result SARS-CoV-2, Ct (FAM™)	**RP result** RP, Ct (JOE™)	**Results interpretation**
+ (Ct≤35)	**+** (Ct<40)	**SARS-CoV-2 detected → POSITIVE**
+ (Ct≤35)	**-** (Ct>40)	**SARS-CoV-2 detected → POSITIVE**
- (Ct>35)	**+** (Ct<40)	**SARS-CoV-2 not detected → NEGATIVE**
- (Ct>35)	**-** (Ct>40)	Invalid test, repeat extraction and repeat test

Note 1: NZYTech recommends repeating the analysis for all samples showing an ambiguous or atypical curve that does not allow a clear interpretation.

Note 2: Interpretation of results must account for the possibility of false negative and false positive results.

• Although the risk of false negative results is mitigated due to the dual target design of the present test, false negative results may be caused by:

- Unsuitable collection, handling and/or storage of samples.
- Sample outside of viraemic phase.
- Failure to follow procedures in this handbook.
- Use of unauthorised extraction kit or real-time PCR platform.

• False positive results may be caused by:

- Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA.
- Unsuitable handling of the positive control SARS-CoV-2 Pos (RdRp & N).
- Unsuitable handling of amplified product (post-amplification plate).

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. In addition, this test cannot rule out diseases caused by other bacterial or viral pathogens.

11. Performance Evaluation

Evaluation of the NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD) performance was carried out on the Applied Biosystem® 7500 FAST, Roche Life Science LightCycler 480 instrument II, Applied Biosystem® QuantStudio 6 Pro, Qiagen Rotor-Gene Q and Bio-Rad® CFX96™ Real Time PCR Systems with additional testing on the Applied Biosystem® StepOnePlus Real Time PCR system. If other equipment is used, the kit should be validated by the user by using previous characterised samples (both positive and negative).

11.1 Expected Results

Typical amplification plots, observed for clinical sample containing SARS-CoV-2 nucleic acids, are presented in Figure 1. The two cases represent examples of clinical samples presenting medium (A) and high (B) SARS-CoV-2 loads. In cases of very high SARS-CoV-2 loads (see Figure 1B) the curve of the JOE™ channel, corresponding to the human RNase P gene, may be absent or display an atypical form.

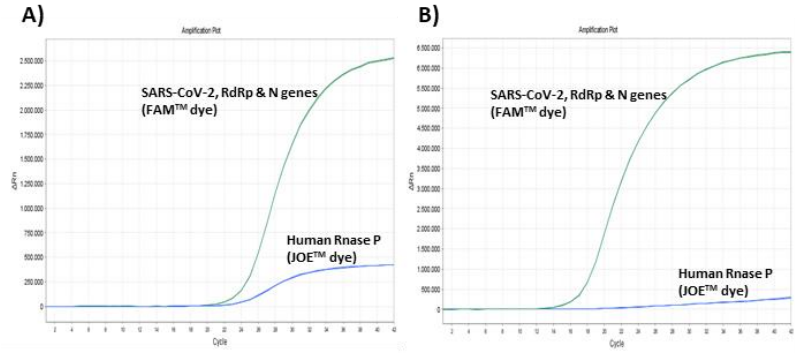

Figure 1. Simultaneous detection of SARS-CoV-2 (RdRp and N genes) and Human RNase-P targets from positive clinical samples with medium (A) and high (B) SARS-CoV-2 loads. Green curves (A & B): detection of the SARS-CoV-2 viral RNA targets (RdRp and N genes) through the FAM channel; Blue curves (A & B): detection of the human RNase P gene through the JOE*channel. *(* VIC/HEX alternative)*

11.2 Limit of Detection (LoD) - Analytical Sensitivity

The analytical sensitivity was defined as the lowest concentration of analyte that could be reliably detected with 95% confidence. This was assessed by testing SARS-CoV-2 nucleic acids at different copy numbers, individually or spiked into RNA extracted from negative oropharyngeal samples, using 3 different kit batches following typical testing reaction conditions. Tests were repeated over 4 days, producing 48 replicates for each SARS-CoV-2 concentration tested. Together, the data revealed that NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD) detects 0.15 copies/µL of SARS-CoV-2 viral RNA with a confidence ≥95%. Thus, the tentative Limit of Detection (LoD) was determined to be 0.15 copies/µL or 150 copies/mL. The tentative LoD was confirmed by two different operators using three kit batches in an experiment with a total of 48 replicates of negative oropharyngeal swab matrix spiked independently. The capacity of NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD) to detect the virus at different loads (from 5 x 10^6 to 5 copies per reaction) is presented in Figure 2.

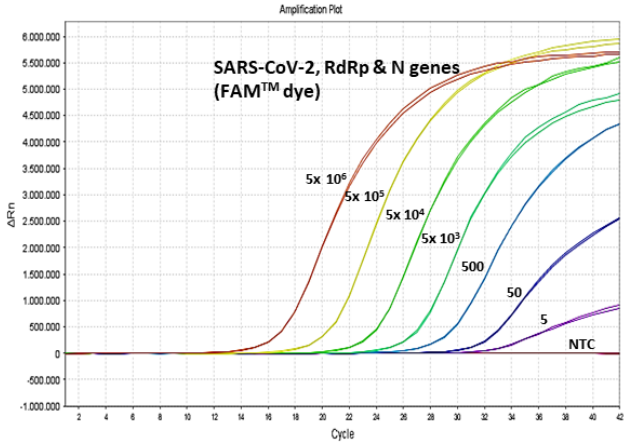

Figure 2. Sensitivity of the SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD). Amplification plot (cycle number *versus* fluorescence - ΔRN) of 1:10 serial dilutions of the SARS-CoV-2 vRNA, from 5 x 10^6 copies to 5 copies per reaction through the FAM™ channel. NTC, No Template Control (negative control).

11.3 Inclusivity, Cross-reactivity and Interfering Substances

Inclusivity and cross-reactivity were evaluated by *in silico* analysis of oligonucleotide probes and primers against pathogens related to SARS-CoV-2 and normal pathogens that cause infection with similar symptoms, respectively. Upon *in silico* analysis the assay design was found to detect all SARS-CoV-2 virus strains and exhibited no reactivity with non-SARS-CoV-2 species.

In vitro analysis for Cross-reactivity (Exclusivity) was performed to confirm that the NZYTech SARS-CoV-2 One-step RT-PCR kit, RdRp and N genes (IVD) (MD0483) does not react with other human flora organisms and the pathogenic organisms that are reasonably to be encountered in the clinical specimen. This study was performed by using three commercial respiratory pathogens panels sourced from ZeptoMetrix Multimarker Controls (#MDZ001), NATtrol™ Respiratory Pathogen Panel-1 (#NATRPP-1) and NATtrol™ RP Controls, (#NATRPC-NNS). These panels that comprise sample polls are representative of the true clinical human specimens, including Influenza A H3N2 (Brisbane/10/07), Influenza A H1N1 (NY02/2009), Rhinovirus Type 1A, Adenovirus Type 3; Parainfluenza Type 1, Parainfluenza Type 2, Parainfluenza Type 3, Parainfluenza Type 4, Metapneumovirus (Peru 6-2003), *Chlamydophila pneumoniae* (CWL-029), *Mycoplasma pneumoniae* (M-129), Coxsackievirus (Type A1), Influenza A H1N1 (A/New Cal/20/99), Influenza A H1N1 (A/Singapore/63/04), Influenza B (B/Florida/02/06), Respiratory Syncytial Virus A, Respiratory Syncytial Virus B (CH93 (18)-18), Coronavirus (HKU-1 recombinant), Coronavirus (OC43), Coronavirus (NL63), Coronavirus (229E), *Bordetella. pertussis* (A639), *Bordetella pertussis* (A747), *Bordetella holmesii* (F061), *Legionella pneumophila* (Philadelphia) and Human Bocavirus. Additionally, other common oral and respiratory tract microbes, including *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, *Burkholderia vietnamiensis*, *Dickeya dadantii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Mycobacterium intracellulare*, *Mycobacterium mageritense*, *Mycobacterium smegmatis*, *Nocardia nova*, *Pseudomonas mendocina*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptomyces avermitilis*, *Streptomyces albidoflavus,* were also tested. None of the organisms tested interfered with NZYTech SARS-CoV-2 One-step RT-PCR kit, RdRp and N genes (IVD) performance by generating false positive result or an unspecific signal.

The impact of 17 potential interferent substances was assessed in tests consisting of negative nasopharyngeal specimens spiked with SARS-CoV-2 positive specimens at ~3x LoD. Potential interfering substances were added to the contrived samples at concentrations representing the highest levels expected in human respiratory patient samples based on literature data. All tests were performed in pentaplicate and results compared to data obtained with a control test that contained no interferents. At the concentrations tested, the results revealed that none of the molecules under test affected the sensitivity of the detection. The table below resumes the data collected under these experiments. All experiments were run on the 7500 FAST Real-time PCR Instrument.

Potential Interferent	Active Ingredient	Final concentration in sample	Interference Yes (Y) or No (N)
Isotonic Sea Water (Rhinomer)	NaCl	15% v/v	N
Throat spray, oral anesthetic and analgesic (Strepfen)	Flurbiprofen	5% v/v	N
Nasal wash solution (Allergy spray - Vibrocil)	Fluticasone propionate	5% v/v	N
Nasal Corticosteroids spray (Nasomet)	Mometasone furoate	5% v/v	N
Nasal Corticosteroids spray (Pulmicort)	Budesonide	5% v/v	N
Antimicrobial, systemic (Trobex)	Trobamycin	600 µg/mL	N
Mouth analgeic, anti-inflamatory and antiseptic (Pyralvex)	Rhubard extract, Salicylic acid	5% v/v	N
Antifungal and Antibacterial Oropharyngeal Topic (Daktarin)	Miconazole	5 mg/mL	N
Mouthwash solution antiseptics (Eludril Gé)	Chlorhexidine gluconate, Chlorobutanol hemihydrate	5% v/v	N
Antitussive, Syrup (Codipront)	Codeine, Phenyltoloxamine citrate	5% v/v	N
Whole Blood (human)	-	4% v/v	N
Antiviral drug (Tamiflu)	Oseltamivir	7,5 mg/mL	N
Mucolytic (Mucolsovan)	Ambroxol hydrochloride	5% v/v	N
Nasal drops solution (Nasarox)	Oxymetazoline Chlorhydrate	10 % v/v	N
Antibiotic, nasal ointment (Bactroban)	Mupirocin	5 mg/mL	N
Saliva (human)	-	25% v/v	N
Absolute ethanol	Alcohol	5% v/v	N

11.4 Precision

Assay precision for the NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD) was determined by the repeated testing of SARS-CoV-2 nucleic acids representing two viral load levels, 5 (1,67x LoD) and 150 (50x LoD) copies per reaction (0.25 and 7.50 copies/µL), individually or spiked into RNA extracted from negative oropharyngeal samples, using 3 different kit batches and following typical testing reaction conditions. Precision was evaluated by measuring Cq average, Cq coefficient of variation and % of replicate detection, as described below for each case. The data is resumed in the table displayed next page.

11.4.1. Repeatability

Repeatability was assessed by one operator by analysing 36 replicates of each sample (5 and 150 copies per reaction), accounting for a final number of 72 tests performed.

11.4.2. Daily Reproducibility

Daily reproducibility was assessed by one operator by analysing 72 replicates of each sample (5 and 150 copies per reaction), for 4 days with 18 replicates of each concentration per day (a total of 144 assays were performed).

11.4.3. Lot-to-lot Reproducibility

Reproducibility between lots was assessed by one operator through the analysis of 144 replicates of each sample (5 and 150 copies per reaction) using 3 different kit batches with 48 replicates per batch.

11.4.4. Operator Reproducibility

Operator reproducibility was assessed by testing 72 replicates of each sample (5 and 150 copies per reaction), by four different operators, with 36 replicates per operator.

11.4.5. Inter-instrument Reproducibility

Inter-instrument reproducibility was measured by one operator through testing 36 replicates of each sample (5 and 150 copies per reaction), in two different qPCR instruments (Applied Biosystem® 7500 FAST, Applied Biosystem® StepOnePlus), in a total of 72 tests per sample.

Precision of NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD)

Variable tested		SARS-CoV-2 (Copies/Reaction)	
		5	**150**
Repeatability			
	n	36	36
	Mean Cq	31,72	26,30
	Coefficient of Variation (%)	1,94	1,60
	% Replicate Detection	100	100
Daily Reproducibility			
	n	72	72
	Mean Cq	31,42	26,13
	Coefficient of Variation (%)	1,50	1,55
	% Replicate Detection	100	100
Lot-to-lot Reproducibility			
	n	144	144
	Mean Cq	31,49	26,24
	Coefficient of Variation (%)	1,61	1,46
	% Replicate Detection	100	100
Operator Reproducibility			
	n	72	72
	Mean Cq	31,45	26,21
	Coefficient of Variation (%)	1,41	1,61
	% Replicate Detection	100	100
Inter-instrument Reproducibility			
	n	72	72
	Mean Cq	31,81	26,46
	Coefficient of Variation (%)	1,61	1,35
	% Replicate Detection	100	100

11.5 Clinical evaluation

The performance of NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD), with collected nasopharyngeal swab samples, was evaluated by two external laboratories. In total, 180 clinical negative and 180 clinical positive samples have been tested. Data revealed that 100% agreement was achieved for all positive and negative samples tested.

12. Quality Control

All components of NZYTech SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes (IVD) are tested following the protocols described above. The triplex real-time PCR system allows the detection of targets described for the identification of SARS-CoV-2 viral RNA (RdRp and N genes) and human mRNA (RNase P gene, RP). Positive amplifications are observed for target genes, positive control and internal controls through FAM and JOE/VIC/HEX channels, according to respective primers/probe set reporter dyes.

13. Technical Support

For Technical support, please contact our dedicated technical support team by Phone: +351 (0) 21 364 35 14 or Email: info@nzytech.com.

14. Trademarks and Disclaimers

All trademarks that appear in this manual are the property of their respective owners.

15. Explanation of Symbols

IVD	*in vitro* diagnostic medical device		Consult instructions for use
REF	Catalogue number		Manufacturer
LOT	Batch code		Use by
	Temperature limitation		Sufficient for
CONTROL +	Positive control		Keep away from the sun light (primer/probe mix)
CONTROL –	Negative control		

16. Conformity Declaration

Product Name: SARS-CoV-2 One-Step RT-PCR Kit, RdRp and N genes, IVD

Catalogue Number: MD04831

Intended use: SARS-CoV-2 qualitative detection.

Manufacturer: NZYTech - Genes & Enzymes,

Estrada do Paço do Lumiar, Campus do Lumiar

Edifício E, R/C,

1649-038, Lisboa

Portugal

We, NZYTech, Lda – Genes & Enzymes, hereby declare that this product, to which this declaration of conformity relates, is in conformity with the following standards and other normative documents ISO 9001:2015, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices as transposed into the national laws of the Member States of the European Union.

The product technical file is maintained at NZYTech, Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal.

Virgínia Pires, PhD

Technical Director

17. References

WHO: Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected. 13 march 2020. Available online at https://www.who.int/docs/default-source/coronaviruse/clinical-management-of-novel-cov.pdf

WHO: Q&A: Influenza and COVID-19 - similarities and differences. 17 March 2020. Available online at https://www.who.int/emergencies/diseases/novel-coronavirus-2019/question-and-answers-hub/q-a-detail/q-a-similarities-and-differences-covid-19-and-influenza

Zhou, Peng; Yang, Xing-Lou; Wang, Xian-Guang; Hu, Ben; Zhang, Lei; Zhang, Wei et al. (2020): A pneumonia outbreak associated with a new coronavirus of probable bat origin. In Nature 579 (7798), pp. 270–273. doi: 10.1038/s41586-020-2012-7.

Chhikara, B. S., Rathi, B., Singh, J., Poonam. (2020). Corona virus SARS-CoV-2 disease COVID-19: Infection, prevention and clinical advances of the prospective chemical drug therapeutics. Chem. Biol. 7(1) 63-72.

Gorbalenya, Alexander E.; Baker, Susan C.; Baric, Ralph S.; Groot, Raoul J. de; Drosten, Christian; Gulyaeva, Anastasia A. et al. (2020): Severe acute respiratory syndrome-related coronavirus: The species and its viruses – a statement of the Coronavirus Study Group (14). bioRxiv 2020.02.07.937862; doi: https://doi.org/10.1101/2020.02.07.937862