MD0319_IFU_en_V2401

SARS-CoV-2 One-Step RT-PCR Kit

Catalogue number	Presentation
MD03191	96 reactions

Description

NZYtech One-Step RT-PCR Kit for detection of novel coronavirus, named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is designed for the *in vitro* quantitative identification of SARS-CoV-2 genome in human biological samples. The kit is build to have the broadest detection profile possible whilst remaining specific to the SARS-CoV-2 genome. It provides the complete set of reagents and probes to detect SARS-CoV-2 genome, including an effective internal control to confirm efficient sample RNA extraction and absence of PCR inhibitors, among others. The virus RNA dependent RNA polymerase (RdRp) gene has been identified as an highly specific marker for SARS-CoV-2. NZYtech kit targets an highly conserved region of SARS-CoV-2 gene, encoding the 3´-5' exoribonuclease domain responsible for the proofreading role for prevention of mutagenesis, through an highly optimized primers/probe set. The primers and probe have 100% homology with >95% of the genome sequences available on the GISAID database. In addition, primers and probe targeting SARS-CoV-2 genome display no significant homology with unrelated genomes rendering this test highly specific as there is no cross reactivity with any other Coronavirus sequenced thus far. 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 revisites SARS-CoV-2 genomic targets and, if required, will release new versions of this kit.

Shipping & Storage Conditions

This product is shipped refrigerated. All components should be immediately stored at -85°C to -15°C upon arrival. Also proceed with the following recommendations:

- Minimise the number of freeze-thaw cycles by storing kit components in working aliquots. The kit is stable for six months from the date of resuspension.
- The PPMix must be stored protected from light. Particularly, do not expose the NZYSpeedy One-step RT-qPCR Master Mix to direct sunlight after combining it with the PPMix.
- If the package that protects the kit arrived damaged, please contact NZYtech.
- Beware of 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.

Components

The kit provides a comprehensive set of reagents sufficient to perform 96 *in vitro* Real-time PCR reactions.

COMPONENT	# TUBES	VOLUME
NZYSpeedy One-step RT-qPCR Master Mix	1	1000 µL
SARS-CoV-2/RP primers/probe Mix (FAM and JOE labelled, respectively)	1	220 µL
SARS-CoV-2/RP Positive Control (1 x 10^4 copies/µL)	1	120 µL
SARS-CoV-2 Copy Number Quantification (1 x 10^7 copies/µL)	1	120 µL
RNase/DNase free water	1	1000 µL

Reagents, Materials and Equipment Required but Not Provided

- Real-time PCR Instrument that detects FAM™ and JOE™ fluorescent dyes (emission wavelengths of 520 and 555, respectively).
- RNA extraction kit: we recommend using NZYtech's RNA extraction kits which are constantly fine-tuned to optimize Molecular Diagnostic applications.
- RNase/DNase-free qPCR plasticware: PCR tubes, strips, caps, 96-well plates, and adhesive films (also available at NZYtech).
- Pipettes and filter tips (RNase/DNase-free).
- Disposable gloves.
- Vortex and centrifuge.

Sample Material

All RNA samples that are suitable for RT-PCR amplification may be used with this kit. However, different factors such as protocol for sample collection from biologic material, transport, storage and processing time are critical to achieve optimal results. Please ensure RNA samples are suitable in terms of purity, concentration and nucleic acid integrity. NZYtech provides 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.

The Dynamic Range of the Test

Under optimal PCR conditions this NZYtech Molecular Diagnostic Real-time RT-qPCR Kit displays high priming efficiencies (>95%) and can detect at least 100 copies of the target template per reaction using different samples.

Rational for the test

One-step RT-qPCR

One-step RT-PCR combines reverse transcription and PCR amplification in a single reaction tube. This saves significant bench time but also reduces errors. The sensitivity of a one-step RT- PCR protocol is also greater than a two-step workflow as the entire biological sample is available to the PCR without dilution. NZYtech NZYSpeedy One-step RT-qPCR Master Mix is a highly efficient and robust reaction mix that was optimized for the efficient amplification of different RNA targets in multiplexing experiments.

Negative Control

To validate any positive findings, a negative control reaction should be included every time the kit is used. To perform this, the RNase/DNase free water should be used instead of RNA template. A negative result for all channels indicates that the reagents have not become contaminated while setting up the run.

Positive control (PC)

The kit includes a Positive control template that allows controlling the performance of RT-PCR reactions. This control corresponds to a synthetic nucleic acid molecule carrying sequences that are homologous to SARS-CoV-2 and RP targeted sequences included in this detection assay. The SARS-CoV-2/RP Positive control should be used directly with the SARS-CoV-2/RP primers/probe mix each time an array of samples is tested. A positive result indicates that the primers and probe sets for detecting the target SARS-CoV-2 and RP genes worked properly in the included master mix in that particular experimental scenario. If a negative result is obtained, the test results are invalid and must be repeated. Care should be taken to ensure that the SARS-CoV-2/RP Positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a post- PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the Positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the Positive control into the positive control well. Each µL of the SARS-CoV-2/RP Positive control contains 1 x 10^4 copies of the SARS-CoV-2 and RP genes (each reaction containing 8 µL of Positive control should measure the presence of 8 x 1 x 10^4 = 8 x 10^4 copies of the target genes).

SARS-CoV-2 Copy Number Quantification

SARS-CoV-2 Copy Number Quantification is used for copy number determination and only contains nucleic acids of SARS-CoV-2 target. This can be used to generate a standard curve of SARS-CoV-2 copy number versus Ct value that will allow deducing copy number in biological samples. SARS-CoV-2 Copy Number Quantification nucleic acid is provided at a concentration of 1 x 10^7 copies of the SARS-CoV-2 per µL.

Standard Protocol

Nucleic Acids Extraction

This kit must be used with RNA isolated from human samples, such as throat swab, nasopharyngeal swab, bronchoalveolar lavage fluid, sputum and environmental samples. The RNA extraction protocol should be performed according to the manufacturer's instructions.

Procedure

1. RT-qPCR reaction mixture

Beware that all pipetting steps and experimental plate set-up should be performed on ice. After the plate is poured proceed 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. Previous 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. **If possible, pipet positive control and standard curve nucleic acids last to avoid contamination events.**

Prepare the RT-qPCR reaction mixture according to the table below that specifies the volumes for 1 and *n* reactions (*n*, number of reactions). NZYtech highly recommends performing qPCR analyses in duplicates, which increases the probability of detection of the pathogen and facilitates the interpretation of results.

COMPONENT	**1 REACTION VOLUME (µL)**	***n* REACTIONS * VOLUME (µL)**
NZYSpeedy One-step RT-qPCR Master Mix	10	*n* x 10
SARS-CoV-2/RP primers/probe mix	2	*n* x 2
Final Volume	**12**	*n* x 12

* Include sufficient reactions for the negative and positive(s) controls. For negative control use NTC.

2. Reaction setup

2.1. Pipette 12 µL of each RT-qPCR mix into individual wells according to your real-time PCR experimental plate setup.

2.2. For the negative control reaction (**mandatory**), add 8 µL of NTC instead of the RNA template into the negative control well. The final volume in each well is 20 µL.
Note 1: *Negative controls should be prepared and properly sealed before the addition of the biological samples and positive controls. If this is not possible, avoid pipetting the negative control in adjacent wells to the positive control and biological samples.*

2.3. For the biological sample(s) reaction(s), pipette 8 µL of each extracted RNA sample into the corresponding wells, according to your experimental plate setup. The final volume in each well should be 20 µL.
Note 2: *Seal all biological samples and negative controls before pipetting the PC into the positive control well. If not possible, avoid pipetting the positive and negative controls and the biological samples in adjacent wells.*

2.4. For the positive control reaction (**mandatory**), add 8 µL of SARS-CoV-2/RP Positive Control (8 x 10^4 SARS-CoV-2 & RP copies) into the corresponding well. The final volume in each well should be 20 µL.

3. Standard Curve Preparation (Optional)

If a standard curve is required for quantitative analysis, proceed according to the described below.

Prepare a reaction mix as follows:

Component	**1 reaction Volume (µL)**	**7 reactions Volume (µL)**
NZYSpeedy One-step RT-qPCR Master Mix	10	70
SARS-CoV-2/RP primers/probe mix	2	14
Final Volume	**12**	**84**

3.1. Standard curve dilution series & set-up:

3.1.1. Pipette 90 µL of RNase/DNase free water into 6 tubes and label them from 2-7.

3.1.2. Pipette 10 µL of SARS-CoV-2 Copy Number Quantification into tube 2.

3.1.3. Vortex thoroughly and spin.

3.1.4. Change tip and pipette 10 µL from tube 2 into tube 3.

3.1.5. Vortex thoroughly and spin.

3.1.6. Repeat steps 3.1.2. (for sequential tubes) to 3.1.5. to complete the dilution series. The final Standard Curves have the following copy number per reaction:

Standard Curve	**Copy number (per µL)**	**Copy number per reaction (per 5 µL)**
Tube 1 - SARS-CoV-2 CNQ	1 x 10^7	8 x 10^7
Tube 2	1 x 10^6	8 x 10^6
Tube 3	1 x 10^5	8 x 10^5
Tube 4	1 x 10^4	8 x 10^4
Tube 5	1 x 10^3	8 x 10^3
Tube 6	1 x 10^2	8 x 10^2
Tube 7	10	80

3.2. Pipette 8 µL of each standard template dilution into each well containing the standard curve reaction mix, according to your experimental plate set-up. The final volume in each well should be 20 µL.

Suggested thermal cycling conditions

NZYSpeedy One-step RT-qPCR Master Mix (2x) is an optimized and highly efficient reaction mixture developed for RT-PCR. The table below displays a standard protocol optimized on a number of platforms. However, these conditions may be adapted to suit different machine- specific protocols.

CYCLES	TEMPERATURE	TIME	NOTES
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*

* Fluorogenic data should be collected during this step through the FAM and JOE channel.

Quality Control

Genomic DNA contamination

The product must comply with internal standards of DNA contamination as evaluated through real-time qPCR.

Nucleases assay

To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the kit component in test for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the kit component in test for 1 h at 37 °C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids. To test DNases or RNases contamination of the nucleic acid controls, dilutions of the controls are incubated for 14-16 h at 37 °C and at -20 °C. After incubation, a qPCR/RT-qPCR reaction is performed comparing Ct values of the samples incubated at 37 °C and at -20 °C. There must be a deviation of less than 2 Cts between the two samples.

Functional assay

The qPCR/RT-qPCR reactions must ensure the consistent amplification of target DNA/RNA and internal extraction control across serial dilutions, meeting specified acceptance criteria for assay performance.

Data analysis

Before analysing sample results, we recommend verifying if the real-time RT-qPCR 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 controls**: the amplification curves of FAM and HEX/JOE/VIC (for SARS-CoV-2 and RP, respectively) are positive. The positive control is expected to amplify with a Cq <28. *Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised. Repeat the test.*
- **Negative control**: no amplification is detected. If the negative control has an amplification curve with a sigmoidal shape, sample contamination may have occurred. *Repeat the test following good qPCR practices.*

After verification of the validity of the test, use the following table for the interpretation of principal results (evaluate the overall shape of the amplification curves; only sigmoidal amplification curves are indicative of true amplification).

Sample Target Cq < 36 (SARS-COV-2 - FAM)	**Sample Target Cq < 40** (RP - JOE)	**Negative Control Cq > 40**	**Positive Control Cq < 28**	**Result**
+	+	-	+	**POSITIVE result for SARS-CoV-2**
+	-	-	+	**POSITIVE result for SARS-CoV-2**
-	+	-	+	**NEGATIVE result**