YOUSEQ

INSTRUCTIONS FOR USE

SARS-CoV-2

MULTIPLEX qRT-PCR KIT

E GENE AND RDRP GENE
100 REACTIONS

CE IVD

REF
YS-qPX-CVD19-002-CE

Issue 1.0
Date: 2021 -07-07

SARS-CoV-2 MULTIPLEX qRT-PCR KIT

For *In Vitro* diagnostic use

100 tests

YS-qPX-CVD19-002-CE

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CONTENTS

1. INTENDED USE

The YouSeq® SARS-CoV-2 Multiplex RT-qPCR kit is an *in vitro* diagnostic (IVD) real-time Reverse Transcriptase PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 COVID-19 in upper respiratory specimens e.g. Nasopharyngeal, Oropharyngeal swab from individuals with signs and symptoms of suspected COVID-19 infection. Testing is limited to laboratories with capability of moderate and high complexity testing by qualified clinical laboratory personnel who are trained to perform the YouSeq® SARS-CoV-2 Multiplex qPCR kit. Extraction of nucleic acid from samples will be undertaken using the QIAamp® Viral RNA Mini extraction method. Positive results indicate presence of SARS-CoV-2 RNA. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for patient management decisions. Positive and Negative results must be combined with clinical observations, patient history, and epidemiological information.

2. BACKGROUND INFORMATION

In December 2019, there was an outbreak of a severe unexplained viral pneumonia in Wuhan City, Hubei Province, China subsequently identified to be a novel coronavirus, SARS-CoV-2. Millions of cases of severe respiratory illness and deaths have been reported worldwide and patients can be infected with SARS-CoV-2 through contact with a contaminated environment or person.

The YouSeq® SARS-CoV-2 Multiplex RT-qPCR kit is a molecular *in vitro* diagnostic test for the qualitative detection of SARS-CoV-2 RNA in upper and lower respiratory specimens from patients suspected of COVID-19. The assay is based on widely used nucleic acid amplification technology and the kit contains oligonucleotide primers, dual-labelled hydrolysis probes and control material for reverse transcription Real-Time PCR.

3. PRODUCT DESCRIPTION

The oligonucleotide primers and probes for specific detection of SARS-CoV-2 are designed to detect regions of RNA-dependent RNA (RdRP) and envelope protein (E) genes of the SARS-CoV-2 genome. The kit includes primers and probes that are specific for the RdRP gene probe labelled with ROX and E gene probe labelled with FAM. The kit also contains primers and probe (labelled with VIC/HEX) for detection of human RNAseP gene as an endogenous internal control for confirming specimen integrity, nucleic acid isolation, amplification, and detection.

Real-Time PCR technology uses polymerase chain reaction (PCR) for the amplification of specific targeted sequences and related probes for the detection of amplified RNA. RNA isolated and purified from upper respiratory tract specimens is reverse transcribed to cDNA and amplified in a Real-time PCR instrument using one-step MasterMix. During the PCR cycle, gene specific probes are hybridised to amplified template and subsequently degraded by the 5′ nuclease activity of Taq DNA polymerase. This causes the reporter dye to separate from the quencher and generate a fluorescent signal that increases with each PCR cycle.

The PCR instrument records a real-time amplification curve for each channel based on fluorescent signal change and calculates quantification cycle (Cq) value to determine the presence/absence of SARS-CoV-2 RNA.

The total length of time from sample swab to result is <120 minutes.

4. MATERIALS PROVIDED

	Volume	Lid Colour
E gene specific primer/probe (FAM) RdRP gene specific primer/probe (ROX) RNAseP endogenous control primer/probe (VIC/HEX)	110 µL	Blue Stripe
Positive Control Template	0.5 mL	Red Stripe
OneStep 2 x qRT-PCR MasterMix	1.0 mL	Green Stripe
RNase/DNase Free Water	1.5 mL	White

5. MATERIALS REQUIRED BUT NOT PROVIDED

- PCR hood
- Benchtop microcentrifuge
- Vortex mixer
- Adjustable micropipettes (2 or 10 µL, 200 µL and 1000 µL)
- Racks for 1.5 mL microcentrifuge tubes
- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- Aerosol barrier pipette tips with filters
- Disposable gloves
- 96 well PCR plate (compatible with BioRad CFX Opus)
- Plate seal
- Ice
- Plate spinner

N.B. The YouSeq Ltd SARS-CoV-2 Multiplex qPCR kit has not been specifically validated on any automated liquid handling platforms.

6. REAL-TIME PCR INSTRUMENTS

The YouSeq Ltd SARS-CoV-2 Multiplex qPCR kit has been fully validated with the following Real-Time PCR instruments:

- CFX Opus™ System (Bio-Rad)

In addition, the following instruments have been shown to be suitable for use with the assay but are not fully validated:

- QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)
- ABI Prism® 7500 SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- Roche® LC480
- CFX96™ System (Bio-Rad)

N.B. please ensure that all instruments used have been installed, calibrated, and maintained according to the manufacturer's instruction and recommendations.

7. REAGENT STORAGE, HANDLING AND STABILITY CONDITIONS

	Shipping	Storage	Expiry Date	Freeze-Thaw
YouSeq® SARS-CoV-2 Multiplex RT-qPCR kit	Kit should be shipped frozen on dry ice. If kit arrives in defrosted state it must be disposed of and not used	-20°C (*+/-5°C*) Stored in the dark The kit should be promptly returned to the freezer after use	Refer to pack label for expiry date The kit should not be used past the "use by" date displayed on the pack label	Keep freeze-thaw cycles to a minimum. YouSeq recommends no more than 5 freeze-thaws

8. SAMPLE TYPES, HANDLING AND STORAGE

Human nasopharyngeal swab specimens from upper respiratory specimens from individuals with signs and symptoms can be tested with the YouSeq Ltd SARS-CoV-2 Multiplex qPCR kit. Inappropriate sampling, storage and transportation may lead to incorrect detection results. The following are recommended:

Collecting the specimen

Refer to Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from Persons under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19)
https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html

- Follow specimen collection devices manufacturer instructions for proper collection methods.
- Swab specimens should be collected using swabs with a synthetic tip, such as nylon or Dracon® and with an aluminium or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 mL of viral transport medium.

Transporting specimen

- Specimens must be packaged, shipped and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. Store specimens at 2-8ºC and ship overnight. If a specimen is frozen at -70ºC or lower, ship overnight on dry ice.

Storing specimen

- Samples may be fresh or frozen. If frozen, samples should not be thawed more than once.
- Samples can be stored at 2–8°C for up to 6 hours.
- Repeated freeze–thawing of swab samples will lead to reduced viral titers and should be avoided for optimal sensitivity.
- For long-term storage, freezing swab samples at –20 to -80°C in aliquots should be practised.

9. SAMPLE PREPARATION

Nucleic acid extraction

The following commercially available RNA extraction kits and procedures have been qualified and validated for recovery and purity of RNA for use with this assay: QIAamp® Viral RNA Mini kit (catalogue number 52904/52906, 50/250 samples). Manufacturer's recommended procedures are to be followed for swab sample viral RNA extraction with a final elution buffer volume of 80µl.

10. qRT-PCR PROCEDURE

n.b. Ensure that Nucleic acid extraction and reaction set up are performed in a designated "pre-PCR" environment free from any DNA/RNA template. Positive Control Template included in the kit should not be opened or stored in this pre-PCR environment.

Equipment preparation

Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. YouSeq Recommendation: Decontamination agents should be used such as 5% bleach, 70% ethanol, and DNAzap™ or RNase AWAY® to minimize the risk of nucleic acid contamination.

Reaction set up overview

The final reaction components are as follows

OneStep 2 x qRT-PCR MasterMix	10 µL
Primer/probe	1 µL
Sample RNA or control template	9 µL

MasterMix and reaction well set up

a. Thaw the OneStep MasterMix, primer/probe and DNase/RNase free water in ambient conditions. Ensure reagents are thawed properly before use.
b. Pulse vortex 3 times and quickly spin down each reagent before use
c. Follow the table below for reaction mix creation.
    i. **N =** Reaction quantity of samples **PLUS** No Template Control (NTC) **PLUS** Positive Control Template (PCT) **PLUS** a known positive sample (KPS)
    ii. All controls mentioned above should be included on every plate
    iii. Overage is applied in the calculation (N + 2)
    iv. Prepare reaction mix based on table below in specified order, in a fresh 1.5 mL Eppendorf

n.b. For optimum sensitivity keep all reagents and plate on ice from hereon and keep set up time to a minimum

	Volume Required		
Component	1 x rxn	N+2 x rxn	e.g., 10 rxns
OneStep 2 x qRT-PCR MasterMix	10 µL	N+2 x 10 µL	120 µL
Target Primer/probe mix	1 µL	N+2 x 1 µL	12 µL
Final Volume	11 µL	N+2 x 11 µL	132 µL

d. Mix the reaction mix before use, by performing 2 pulse vortexes and quickly spin down
e. Use a 96-well plate suitable for use with your real-time PCR instrument
f. Pipette 11 µL of the reaction mix into the specified wells, required for your testing and controls (1 well for the PCT, 1 well for the NTC, 1 well for KPS)
g. Pipette 4 µL of RNase/DNase free water into the Positive Control well
h. Pipette 9 µL of RNase/DNase free water into the No Template Control well

n.b. Ensure that template is only opened, prepared, and added to plate in a designated "post-PCR" environment.

1. Add template
    a. Pipette 9 µL of extracted RNA samples into specified PCR wells
    b. Pipette 9 µL of known positive RNA samples into specified PCR
    c. Pulse vortex the PCT 3 times and quickly spin down
    d. Pipette 5 µL of positive control template into the PCT well
    e. Seal the plate securely with a suitable adhesive Optical PCR seal
    f. Pulse spin plate in plate spinner to remove any air bubbles

2. Programming the Real-Time PCR instrument

Use the following thermal cycling parameters:

	Temp	Time
Reverse Transcription	55°C	10 minutes
Hot start	95°C	3 minutes
45 cycles	95°C	15 seconds
	60°C*	60 seconds

n.b. Ensure that data collection is made through the FAM, ROX and VIC/HEX channels during this annealing phase.

n.b. YouSeq recommends that after the run is complete that the plate is sealed in a "zip seal" plastic bag and disposed of to minimise any risk of laboratory contamination with PCR product.

11. DATA INTERPRETATION

ROX passive reference dye

The reaction mix does not contain ROX passive reference dye. Ensure that ROX passive reference dye is switched "off" in the software analytical settings.

Check validity of diagnostic run

Positive control

Check the positive control performance. The positive control should amplify in a Cq range of approximately 18.5 +/- 2

Known Positive Sample

Check the Known Positive Sample performance. The KPS should amplify in a Cq range <40

Negative control

The negative control well should deliver a flat line up to 45 cycles i.e., negative result.
If a positive Cq value < 45 cycles is achieved, an investigation of background laboratory contamination should be performed to determine the source of template

The test is valid if the following conditions are met:

	FAM channel	ROX channel	HEX channel
Positive control	+	+	-
Known Positive	+	+	-
Negative control	-	-	-

If these conditions are not met the test should be repeated

Positive samples

Samples that are positive for the pathogen will deliver a defined "sigmoidal" amplification plot.

RNAseP endogenous PCR control

The Cq value obtained with the endogenous control will confirm successful extraction of nucleic acid and quality of the biological material. Detection of the endogenous control is through the VIC/HEX channel.

Detection of the endogenous control is not required for positive results either in the FAM detection channel or in the ROX detection channel. A high B-βCoV (E gene) and/or SARS-CoV-2 (RdRP gene) RNA load in the sample can lead to reduced or absent endogenous control signals.

Qualitative analysis

	qRT-PCR results				
Target E gene (FAM)	+	-	+	-	-
Target RdRP gene (ROX)	+	+	-	-	-
RNAseP endogenous control (HEX/VIC)	+/-	+/-	+	+	-
Result	**Positive result** B-βCoV and SARS-CoV-2 specific RNA detected. Positive for SARS-CoV-2.	**Positive result.** Only SARS-CoV-2 specific RNA detected. Positive for SARS-CoV-2	Only B-βCoV specific RNA detected. Presumptive positive for SARS-CoV-2*	**Negative result.** Neither B-βCoV nor SARS-CoV-2 specific RNA detected. The sample does not contain detectable amounts of SARS-CoV-2 specific RNA.	Failed test. Repeat required

* Sample may be re-tested by repeating the extraction and qRT-PCR. If the repeated result remains presumptive positive for SARS-CoV-2 then additional confirmatory testing may be conducted.

APPENDICES

12. PERFORMANCE EVALUATION

Analytical Sensitivity

Result: 0.91 copies per µL in reaction

The YouSeq® SARS-CoV-2 Multiplex RT-qPCR kit has an analytical sensitivity of 0.91 copies of SARS-CoV-2 RNA genome per µL, in a reaction.
Analytical sensitivity was determined by probit analysis.

Input Conc. Copies per/ µl in a Reaction	No of replicates	No of positives	Hit rate %
20	21	21	100%
10	21	21	100%
5	21	21	100%
2	21	21	100%
1	21	21	100%
0.4	21	20	95.2%

Analytical Specificity

The analytical specificity of the YouSeq® SARS-CoV-2 Multiplex RT-qPCR kit is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant variants and genotypes will be detected.

Inclusivity

Inclusivity of the YouSeq® SARS-CoV-2 Multiplex RT-qPCR kit was evaluated for SARS-CoV-2 by wet lab testing SARS-CoV-2 sample material from NIBSC (Cat no. 19/304) and Amplirun® SARS-CoV02 RNA control material from Launch Diagnostics (Cat no. MBC137-R)

Inclusivity (*In silico* analysis for 1,450,073 whole genome sequences of SARS- CoV-2 published via GISAID e.V. (www.gisaid.org) as of June 18, 2021 for the E gene and the RdRp gene targets

258,855 Whole genome sequences		Homology	Comment
E gene	Forward primer	1,442,053 (99.4 %)	All single base mismatches identified in the E primers were towards the 5' end (outside of the last 6 bases) and will not affect detection.
	Reverse primer	1,446,226 (99.7 %)	
	Probe	1,442,220 (99.4 %)	
RdRp gene	Forward primer	1,353,106 (93.2 %)	>99.9 % of single base mismatches identified in the RdRP primers were towards the 5' end (outside of the last 6 bases) and will not affect detection, All mismatches identified within the RdRp reverse primer were present in a small group of sequences originating from the USA between May 2020 and August 2020 and are likely part of a failed lineage. Identified mismatches will not affect inclusivity performance.
	Reverse primer	1,446,751 (99.7 %)	
	Probe	1,436,491 (99.0 %)	

Cross Reactivity

The analytical specificity of the YouSeq® SARS-CoV-2 Multiplex RT-qPCR kit with respect to cross reactivity with pathogens other than SARS-CoV-2 was evaluated by testing viruses related to SARS-CoV-2, pathogens causing similar symptoms as an infection with SARS-CoV-2 and pathogens likely to be present in patients suffering from a SARS-CoV-2 infection.

YouSeq SARS-CoV-2 Multiplex qRT-PCR kit did not cross-react with any of the following pathogens:

Influenza A H1N1
Influenza A H3
Influenza A 2009 H1N1
Influenza B
Metapneumovirus 8
Respiratory Syncytial Virus A
Rhinovirus 1A
Parainfluenza type 1
Parainfluenza type 2
Parainfluenza type 3
Parainfluenza type 4
Adenovirus Type 3
Coronavirus NL63
Coronavirus 229E
Coronavirus OC43
Coronavirus HKU-1
M. pneumoniae
C. pneumoniae
B. pertussis

Precision

Precision of the YouSeq® SARS-CoV-2 Multiplex RT-qPCR kit was determined as repeatability (variability within one experiment), reproducibility (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the 3 analyses.
The variability data are expressed in terms of standard deviation and coefficient of variation based on quantification cycle (Cq) values. At least 4 replicates per sample were analysed for intra-assay variability, inter-assay variability and inter-lot variability.

		COVID19 E-gene Result (FAM)			COVID19 RdRP-gene Result (ROX)		
		Mean	Std Dev	Coefficient of Variation (%)	Mean	Std Dev	Coefficient of Variation (%)
High Sample	Inter-assay variability	34.4	0.54	1.61	34.5	0.42	1.24
	Intra-assay variability		0.44	1.28		0.33	0.95
	Inter-lot variability		0.67	2.12		0.74	1.15
Med Sample	Inter-assay variability	35.6	0.77	2.22	35.9	0.63	1.81
	Intra-assay variability		0.65	1.82		0.48	1.33
	Inter-lot variability		1.02	3.12		0.86	2.54
Low Sample	Inter-assay variability	35.7	0.84	2.38	36.2	0.71	1.98
	Intra-assay variability		0.54	1.52		0.60	1.67
	Inter-lot variability		0.98	3.01		0.54	2.87

Diagnostic Evaluation

The YouSeq® SARS-CoV-2 Multiplex RT-qPCR kit was evaluated in a comparative study with the CE-marked SARS-COV-2 RT-PCR kit (VIASURE). Retrospectively, 50 contrived SARS-CoV-2 positive samples were tested in parallel using the SARS-COV-2 RT-PCR kit (VIASURE) and the YouSeq® SARS-CoV-2 Multiplex RT-qPCR kit in combination with the OPUS™ System (Bio-Rad).

		Comparative Method (VIASURE SARS-CoV-2 Kit)		
		Pos	Neg	Total
Candidate Method (YouSeq SARS-CoV-2 Kit)	Pos	50	0	50
	Neg	0	50	50
	Total	50	50	100

The diagnostic sensitivity and specificity of the YouSeq® SARS-CoV-2 Multiplex RT-qPCR kit compared to the SARS-COV-2 RT-PCR kit (VIASURE) were 100 % and 100 %, respectively.

13. SYMBOLS

Symbol	Explanation
IVD	*in vitro* diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer

12. QUALITY CONTROL

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of COVID-19 2 gene multiplex qRT-PCR kit is tested against predetermined specifications to ensure consistent product quality.

13. TECHNICAL ASSISTANCE

For customer support or to provide feedback, please contact our Technical Support:

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phone: +44 (0)333 577 6697

14. TRADEMARKS AND DISCLAIMERS

YouSeq®, ABI Prism® (Applied Biosystems); OPUS, CFX96™ (Bio-Rad); Rotor-Gene®, QIAamp®, QIAsymphony® (QIAGEN); LightCycler® (Roche); FAM™, ROX™ (Thermo Fisher Scientific), Amplirun® (Vircell).

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Not available in all countries.

15. WARNINGS, PRECAUTIONS AND LIMITATIONS

- For in vitro diagnostic use (IVD) only.
- Do not use if passed use by date
- This test has been authorized only for the detection of nucleic acids from SARS-CoV-2, not for any other viruses or pathogens.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html
- Specimen processing should be performed in accordance with national biological safety regulations.
- Perform all manipulations of potential live virus samples within a class II (or higher) biological safety cabinet. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Use personal protective equipment such as (but not limited) gloves, eye protection and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes and other equipment and reagents.
- Please consult the material safety data sheet (MSDS) before using this kit, which is available on request.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon).
- The YouSeq SARS-CoV-2 positive control template is provided in a sealed plastic pouch and contains a high copy number of templates. It should be opened and processed away from test samples and kit components to avoid cross-contamination.
- Maintain separate areas for handling of specimen preparation, pre-PCR assay setup and post-PCR amplified nucleic acids.
- Maintain separated, dedicated equipment (e.g. pipettes, microcentrifuge) and supplies (e.g. microcentrifuge tubes, pipette tips) for handling of specimen preparation, pre-PCR assay setup and post-PCR amplified nucleic acids.
- Wear a clean lab coat and disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- Good aseptic technique should always be used when working with nucleic acids.
- Work surfaces, pipettes and centrifuges should be cleaned and decontaminated with cleaning products (e.g. DNA/RNA remover, ethanol) to minimize risk of nucleic acid contamination.
- RNA should be maintained on a cold block or on ice during preparation and use to ensure stability.
- After each run has been set up and performed, clean work surfaces and equipment with a DNA/RNA remover.
- Handle post-amplification plates with care to ensure that the seal is not broken.
- Dispose of unused kit reagents and human specimens according to local, state and federal regulations.
- Work swiftly and on ice.
- YouSeq OneStep qRT-PCR MasterMix contains a powerful reverse transcriptase enzyme to deliver maximally efficient conversion of viral RNA to cDNA. This enzyme is active at room temperature.
- If left at room temperature in the presence of primers/probes the Reverse Transcriptase can react with the primers and probe to cause artefacts that reduce assay sensitivity.
- Therefore, it is critical to store your primer/probe and MasterMix reaction mix on ice and for periods of no more than 30 minutes.
- For the same reason, set up your qPCR reaction plate on ice and proceed to amplification within 30 minutes. Do not delay.