YOUSEQ

SARS-CoV-2 MULTIPLEX qPCR KIT USER GUIDE

CAT NO. YSL-qPX-E_N_RdRp-CVD19-100

100 reactions
With Endogenous Control and Lyophilised MasterMix

VERSION 4.0

For Research Use Only

YOUSEQ

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INTENDED USE

This product is a qPCR test kit for the detection of SARS-CoV-2 (COVID-19) RNA in good quality nucleic acid samples from a variety of sources. It is designed to be used by trained users in a suitable molecular biology laboratory environment.

KIT CONTENTS

	Cap colour	Volume
E gene primer/probe (FAM Probe) RdRp gene primer/probe (ROX Probe) N gene primer/probe (Cy5 Probe) RNaseP primer/probe (VIC/HEX Probe)		110 µl
Positive Control Template		500 µl *
Lyophilised Tetra OneStep 2X qRT-PCR MasterMix		1.1 ml *
MasterMix resuspension buffer		1.5ml
Template resuspension buffer		1.5ml
DNase/RNase free water		1.5ml

* Supplied lyophilised and requires resuspension, see resuspension step below for instructions

RESUSPENSION STEP

Resuspend the kit contents with the correct reagents as per the table below. Spin or gently tap all tubes and vials to ensure all the contents are at the bottom of the tube.

After adding the resuspension reagent, pulse, or vortex the vials again to ensure it is mixed well.

	Reagent	Volume
Positive Control Template	Template resuspension buffer	500 µl
Lyophilised Tetra OneStep 2X qRT-PCR MasterMix	MasterMix resuspension buffer	1.1 ml

MATERIALS REQUIRED BUT NOT PROVIDED

DNA/RNA Extraction Kit – This kit will work well with any RNA extraction kit that yields high quality RNA with minimal PCR inhibitors present.

qPCR instrument with minimum 4 colour detection (FAM, ROX, Cy5, VIC/HEX).

Pipettes and general laboratory equipment.

KIT SPECIFICITY

The YouSeq qPCR test kit for detection of SARS-CoV-2 (COVID-19) is designed to have the broadest detection profile possible and detect all clinically relevant strains. The primers and probes have very high (>95%) homology with all reference data within the NCBI database.

The target genes (E gene, N gene and RdRp gene) have been demonstrated to have unique sequences within the species making them ideal targets for highly specific detection of all publicly available SARS-CoV-2 sequences and no closely related viruses.

If you require more specific data about the detection profile of the kit, please do not hesitate to contact our bioinformatics team: support@youseq.com

qPCR BENCH SIDE PROTOCOL

Clean and decontaminate all work surfaces, pipettes, and other equipment prior to use to remove potentially contaminating nucleic acids.

REACTION SET UP

Combine the following reagents to create a final test reaction:

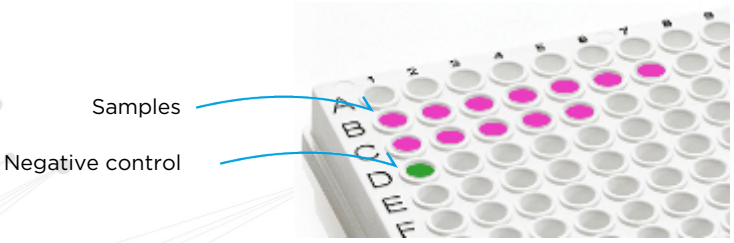
Component	Volume
Tetra OneStep 2X qRT-PCR MasterMix	10 µl
Primer/probe mix	1 µl
Sample RNA	9 µl
Final Volume	20 µl

Please note: Work swiftly and on ice. Tetra OneStep qRT-PCR MasterMix contains a powerful reverse transcriptase enzyme to deliver maximally efficient conversion of viral RNA to cDNA. This enzyme is partially 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.

NEGATIVE CONTROL

For a negative control reaction, repeat the reaction set up above replacing the sample RNA with DNase/RNase free water.



Please note: Make sure to seal the sample and negative control wells before proceeding to the positive control step.

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POSITIVE CONTROL

For a positive control reaction, repeat the reaction set up above but replace the extracted sample RNA with 9 µL of the resuspended positive control template supplied with the kit.

qRT-PCR AMPLIFICATION PROTOCOL

Run the following PCR protocol:

Please note: If using a qPCR machine that uses ROX as a passive reference, then the passive reference must be turned off or set to "none" indicating no passive reference.

	Temperature	Time
	55°C	10 minutes
Hot Start	95°C	3 minutes
45 cycles	95°C	15 seconds
	60°C*	60 seconds

*Data collection for appropriate target channels – FAM, ROX, CY5, VIC/HEX

INTERPRETATION OF RESULTS

When analysing Sample Cq values, YouSeq recommends checking the threshold within the run file before interpreting the data. We would suggest setting the threshold to 10% of the relevant positive control End Point Fluorescence (EPF).

Positive Control

Firstly, check the positive control performance. The positive control should amplify in a Cq range of approximately 18+/-2.
The test is valid if the following conditions are met:

	FAM channel	ROX channel	Cy5 Channel
Positive control	+	+	+
Negative control	-	-	-

Please note: The positive control in the kit is a representative sequence associated to the designs target region and does not contain the organism's entire genome.
The positive control within the kit does not include the endogenous control sequence. Therefore, the positive control should not be expected to amplify in the endogenous control channel.

Negative Control

In ideal circumstances, the negative control well should deliver a flat line – negative result. However, it is not uncommon for background laboratory contamination to cause a very late signal. If this signal is ≥5 Cq values later than your sample signal, then it can be considered negative, and the result is viable.
If the negative is <5 Cq later than the sample result, then the result is inconclusive, and the test should be repeated after potential sources of contamination have been removed.

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Positive Samples

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

Endogenous 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 RNaseP 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 or Cy5 detection channel. A high B-βCoV (E gene) and/or SARS-CoV-2 (RdRp or N gene) RNA load in the sample can lead to reduced or absent endogenous control signals.

Results interpretation:

	qPCR results				
Target E gene (FAM)	+	-	+	-	-
Target RdRp gene (ROX) and N gene (Cy5)	+	+	-	-	-
RNaseP control (HEX/VIC)	+/-	+/-	+	+	-
Result	**Positive result** B-βCoV and SARS-CoV-2 specific RNA detected.	**Positive result.** Only SARS-CoV-2 specific RNA detected.	**Presumptive positive.** Only B-βCoV specific RNA detected.	**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

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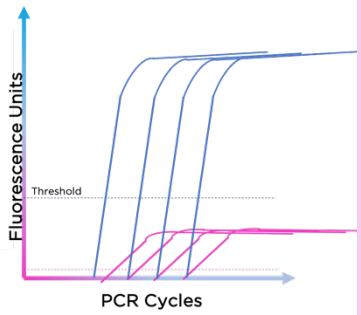
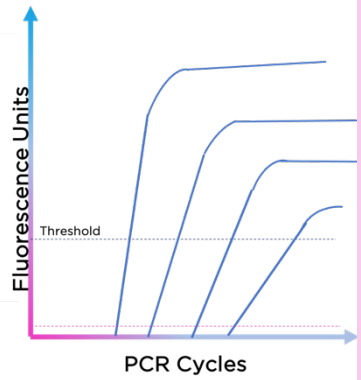
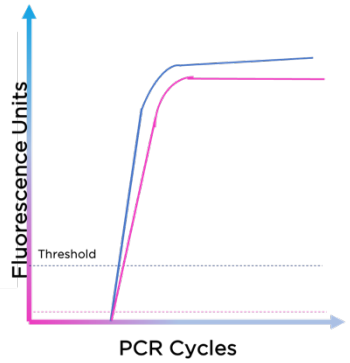
MULTIPLEX TROUBLESHOOTING

	Trace	What can you see?	Cause	Action
1		One assay with greater end point fluorescence than another	Some fluorophores are brighter than others. Also, certain instruments detect different fluorophores with higher/lower efficiency	Analyse each channel individually so the Y-axis is correct for each fluorophore. or Analyse on logarithmic scale instead of linear scale
2		Traces for weak positives (with later Cq values) appear “leant over” or “flatter” without strong sigmoidal curve	Artefact formation typically driven by Reverse Transcriptase	Minimise the time primer/probe spends in MasterMix during plate set up. Store reagents and set up plate on ice/cold block during experiment set up. Move swiftly to complete plate set up and commence qPCR after plate set-up
3		Amplification/ unusual Cq value in unexpected fluorescent channel. Cq value/curve shape very similar to adjacent fluorescent channel	‘Bleed through’ or ‘cross talk’ between channels. Amplification from one fluorescent channel has been mistakenly identified in its adjacent channel (e.g., FAM identified as HEX)	Ensure manufacturer recommends the dye combination used in this kit. Recalibrate qPCR instrument.

PRODUCT SPECIFICATIONS

How sensitive is my kit?

The kit is suitable for the detection of SARS-CoV-2 (COVID-19), across a wide dynamic range from >106 copies to less than 100 copies of the target amplicon in the PCR reaction.

Storing your kit

Store at -20°C from arrival. The qPCR kits shelf life is outlines as an expiry date on the pouch label.
Once you have prepared the positive control it can be stored frozen.

Use good quality RNA

Poor quality input nucleic acid is the biggest cause of test failure. The kit will work well with any source of good quality RNA. Good quality is defined as RNA with high integrity (not degraded) and with low levels of inhibitors present.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use. It should not be used for diagnosis of disease unless specifically approved by the regulatory authorities in the country of use.

Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of SARS-CoV-2 Multiplex is tested against predetermined specifications to ensure consistent product quality. Design of the kit met our robust bioinformatic analysis requirements resulting in a clinically relevant detection profile based on available sequence information. The kit is periodically checked against newly available sequence information to remain clinically relevant.

Technical Assistance

For customer support, please contact:

e-mail: support@youseq.com
phone: +44 (0)333 577 6697

Trademarks and Disclaimers

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Not available in all countries.