User Guide

YOUSEQ

SARS-CoV-2 Multiplex qPCR kit

E gene and RdRp gene

100 reactions
(Lyophilised format)

Version 1.3

For Research Use Only

Introduction

This kit employs a Multi target strategy to detect **SARS-CoV-2 (COVID-19)** making for fast efficient diagnosis of the virus. Primers/probe are shown *in silico* to detect all publicly available **SARS-CoV-2** sequences and no closely related viruses.

Kit Contents

	Volume/Rxns
E gene specific primer/probe (FAM Probe) **RdRp gene specific primer/probe** (ROX Probe) **RNaseP control primer/probe** (VIC/HEX Probe)	100 rxns
Positive control Template	50 preps
Template Resuspension Buffer	1.5 ml
Lyophilised OneStep 2 x qRT-PCR MasterMix	2 x 50 rxns
Lyophilised Mastermix resuspension buffer	1.5 ml
RNase/DNase free Water	1.5 ml

Resuspension step

Resuspend the kit contents with the correct reagents as per this table:

	Reagent	Volume
Primer/probe	RNase/DNase free water	110 µl
Positive control Template	Template Resus. Buffer	500 µl
Lyophilised OneStep 2 x qRT-PCR MasterMix	Lyophilised Mastermix resuspension buffer	600µl per vial

qRT-PCR detection protocol

Combine the following reagents to create a test reaction:

Component	Volume
2x OneStep MasterMix	10 µL
Target Primer/probe mix	1 µL
Sample RNA	9 µL
Final Volume	20 µL

Please note. Set up your qPCR reaction plate on ice and proceed to amplification quickly. Prolonged incubation of the reaction mix, particularly at room temperature, can reduce the assay sensitivity.

Negative control

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

Positive control

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

qRT-PCR amplification protocol

This YouSeq kit will work with any qPCR instrument capable of detecting FAM, ROX and VIC/HEX. Use the following cycling conditions:

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

	Temp	Time
	55°C	10 minutes
	95°C	3 minutes
40 cycles	95°C	15 seconds
	60°C*	60 seconds

*Data collection for appropriate target channels-. **FAM, ROX, VIC/HEX**

Interpretation of results

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
Positive control	+	+
Negative control	-	-

Negative control

In ideal circumstances, the negative control well should deliver a flat line – negative result. n.b. Be vigilant. It is not uncommon for background laboratory contamination to cause a very late signal.

Positive samples

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

RNaseP 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 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 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.

	qPCR results				
Target E gene (FAM)	+	-	+	-	-
Target RdRp gene (ROX)	+	+	-	-	-
RNaseP 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 RT-PCR. If the repeated result remains presumptive positive for SARS-CoV-2 then additional confirmatory testing may be conducted.