User Guide

SARS-CoV-2 qPCR kit

S Gene
(Lyophilised kit format)

100 reactions

Version 1.2

For Research Use Only

Introduction

This kit employs a simple single target strategy to detect the SARS-CoV-2 (S gene) making for fast efficient diagnosis of the virus. Primers/probe are shown *in silico* to detect all publicly available nCoV 2019 sequences.

Kit Contents

	Volume/Rxns
S Gene specific (FAM Probe) & RNaseP control (VIC/HEX Probe) primer/probe mix	100 rxns
Positive control Template	50 preps
Template resuspension Buffer	1ml
Lyophilised OneStep 2 x qRT-PCR MasterMix	2 x 50 rxns
Lyophilised Mastermix resuspension buffer	1.5ml
RNase/DNase free Water	1ml

Resuspension step

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

	Reagent	Volume
Target specific primer/probe	RNase/DNase free water	110 µl
RNaseP control 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
OneStep MasterMix	10 µl
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 replacing the sample RNA with RNase/DNase free water.

Positive control standards

For a negative control reaction, repeat the reaction set-up above, replacing the sample RNA with 9µl 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 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, VIC/HEX

Interpretation of results

Positive control

Firstly, check the positive control performance. The first point of your standard curve should amplify in a Cq range of approximately 18+/-2. Amplification outside of this range suggests a failure and the test should be repeated.

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 away from your sample signal, then it can be considered negative and the result is viable.
However, if the negative control is <5 Cq away from your sample result then the result is inconclusive and should be repeated.

Positive samples

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

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

	qPCR signal							
Target Pathogen	≤30	>30	>30	-	+/-	+/-	-	+/-
Positive control	+	+	+	+	+	+	+	-
Endog control	+/-	+	-	+	+/-	+/-	-	+/-
Negative control	-	-	-	-	≤33	>33	-	+/-
Result	Quantitative result positive	Quantitative result positive	Qualitative result positive	Negative result	Failed test (contamination)	See negative ctrl advice above	Failed test. Insufficient target	Failed Test