User Guide

YOUSEQ

SARS-CoV-2 qPCR kit

WHO Methodology
RdRP gene, E gene, N gene

(Lyophilised kit format)

100 reactions

Version 1.2

For Research Use Only

Introduction

This kit employs a thorough 3 target strategy to detect SARS-CoV-2 as recommended by the WHO thus making for highly robust diagnosis of the virus.

Kit Contents

	Volume/Rxns
E gene specific primer/probe (FAM Probe)	100 rxns
RdRp gene specific primer/probe (FAM Probe)	100 rxns
N specific primer/probe (FAM Probe)	100 rxns
Endogenous control primer/probe (VIC/HEX Probe)	100 rxns
Positive control Template	500 µl
Template Resuspension Buffer	1.5 ml
Lyophilised OneStep 2 x qRT-PCR MasterMix	6 x 50 rxns
Lyophilised Mastermix resuspension buffer	3x 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
4 x Primer/prob sets	RNase/DNase free water	110 µl each
Positive control Template	Resus. Buffer	500 µl
Lyophilised OneStep 2 x qRT-PCR MasterMix	Lyophilised Mastermix resuspension buffer	600µl per vial

qPCR detection protocol

For each sample set up 3 reactions, one for each target gene, according to the table below

Component	Volume
OneStep MasterMix	10 µL
Target Primer/probe mix	1 µL
Endogenous control primer/probe mix	1 µL
Sample RNA	8 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

The positive control supplied, contains calibrated positive template for all three targets. 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.

qPCR amplification protocol

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 (e.g. FAM, VIC/HEX, etc)

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 target genes of interest will deliver defined "sigmoidal" amplification plots.

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							
All three Target genes	≤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