

# User Guide



YOUSEQ

## SARS-CoV-2 qPCR kit

E 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 (E 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 |
|---|-------------|
| E 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 $\mu$ l |
| Primer/probe mix  | 1 $\mu$ l  |
| Sample RNA        | 9 $\mu$ l  |
| Final Volume      | 20 $\mu$ l |

**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 9 $\mu$ l 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       |
|-----------|-------|------------|
| 40 cycles | 55°C  | 10 minutes |
|           | 95°C  | 3 minutes  |
|           | 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 |