RT-qPCR Kit

EXTRACTION-FREE CHEMISTRY FOR THE DETECTION OF VIRAL RNA

The RT-qPCR Kit* is optimized for one-step real-time reverse transcriptase PCR (RT-qPCR) quantification of RNA templates, including total RNA and viral RNA (e.g. SARS-CoV-2 viral RNA) from a wide range of targets via probe detection. The one-step master mix uses proprietary reverse transcriptase technology and buffer chemistry for efficient cDNA synthesis and qPCR in a single tube. The kit provides high specificity and sensitivity in simplex and multiplex detection, making it the choice for low-copy-number targets in pathogen detection.

The RT-qPCR master mix is supplied as a 5× premix in a lyophilized format to facilitate easy preparation of reaction mixtures and allow for shipment and storage at room temperature without the need for refrigeration.

*For research use only

Features

- Stable enzyme and mix for room-temperature shipment andstorage
- Convenient master mix for detection of low-copy viral pathogen targets
- High specificity and sensitivity across a wide range of sample sources
- For research use only (RUO)

Applications

- One-step RT-qPCR from RNA templates including viral RNA
- Robust master mix suitable for use with standard and fast qPCR platforms

Fig 1. The RT-qPCR Kit includes one-step master mix and buffer for one-step RT-qPCR from RNA templates including viral RNA.

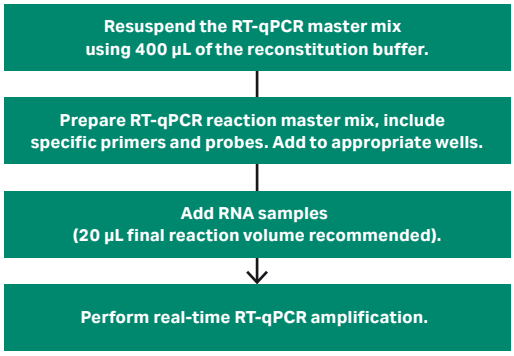

Fig 2. One-step RT-qPCR amplification workflow using the RT-qPCR Kit.

Limit of detection: reliable detection of SARS-CoV-2 viral RNA down to 1 copy/µL

The RT-qPCR Kit has been optimized to provide high specificity and wide dynamic range in one-step RT-qPCR. Synthetic viral RNA (Microbiologics, 9009) covering the nucleocapsid gene of SARS-CoV-2 was used to assess the performance and define the approximate limit of detection of the RT-qPCR Kit in an experimental setting relevant to COVID-19 detection. Viral particles were spiked at between 1 to 10 000 copies/µL into nuclease free water. Un-spiked nuclease free water (no viral RNA) served as the assay control to eliminate the possibility of false positives. One-step reverse transcription and amplification reactions were carried out using the following thermal cycling conditions: 50°C for 10 minutes and 95°C for three minutes, followed by 45 cycles of 95°C for 10 seconds and 60°C for 30 seconds. The RT-qPCR Kit was tested using Centers for Disease Control and Prevention (CDC) Novel Coronavirus (2019-n-CoV) Diagnostic Panel primers (N1 and N2 targeting two regions of the SARS-CoV-2 nucleocapsid gene, Integrated DNA Technologies) in simplex format.

Samples were run in technical triplicate in three independent experiments. As per CDC guidelines, only samples for which both markers (N1 and N2) crossed the threshold at Ct < 40 were considered positive. No amplification in any of the SARS-CoV-2 specific primer sets were observed in un-spiked controls (data not shown).

The results presented in Figure 3 confirm the RT-qPCR Kit is robust and sensitive enough for reproducible detection of low viral titer down to 1 copy/µL in the input sample and demonstrates excellent PCR linearity across an input range of 5 orders of magnitude.

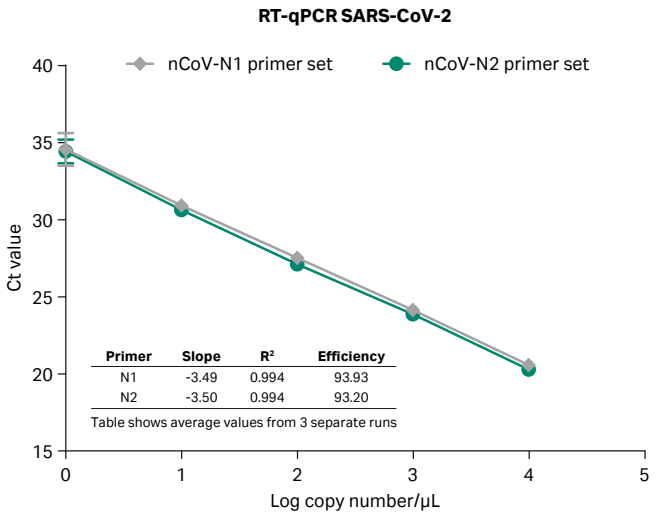

Fig 3. Limit of Detection (LOD) data for the RT-qPCR Kit following RT-qPCR amplification of varying amounts of viral synthetic RNA spiked at between 1 and 10 000 copies/µL in the input sample. Ct values averaged from three independent experiments, n = 3 samples per experiment. Error bars represent standard deviation.

Detection of SARS-CoV-2 viral RNA extracted using the Sera-Xtracta™ Virus/Pathogen Kit

Performance of the RT-qPCR Kit was assessed for the detection of viral RNA isolated using the Sera-Xtracta™ Virus/Pathogen Kit (Cytiva, 29506009, 29514201) in an experimental setting relevant to COVID-19 detection from human swab samples.

Briefly, synthetic viral RNA covering the nucleocapsid gene of SARS-CoV-2 was spiked at between 1 to 100 copies/µL into a diluent consisting of a suspension of human cells (~ 3 × 10^5/mL) in 200 µL viral transport medium (VTM)[†] to mimic a clinical sample (1). Un-spiked cell suspension (no viral RNA) served as the assay control to eliminate the possibility of false positives. The samples were processed using Sera-Xtracta™ Virus/Pathogen Kit following the manufacturer's protocol and eluted in 50 µL of nuclease-free water. Extracted samples (5 µL of eluant per well) were subjected to RT-qPCR as previously described using CDC 2019 Novel Coronavirus (2019-nCoV) Diagnostic Panel primers (N1 and N2). All samples were run in technical triplicates.

The results, presented in Figure 4, confirm that the RT-qPCR Kit is robust and sensitive enough for reproducible detection of low viral titer down to 1 copy/µL in the input sample from viral RNA samples isolated using the Sera-Xtracta™ Virus/Pathogen Kit.

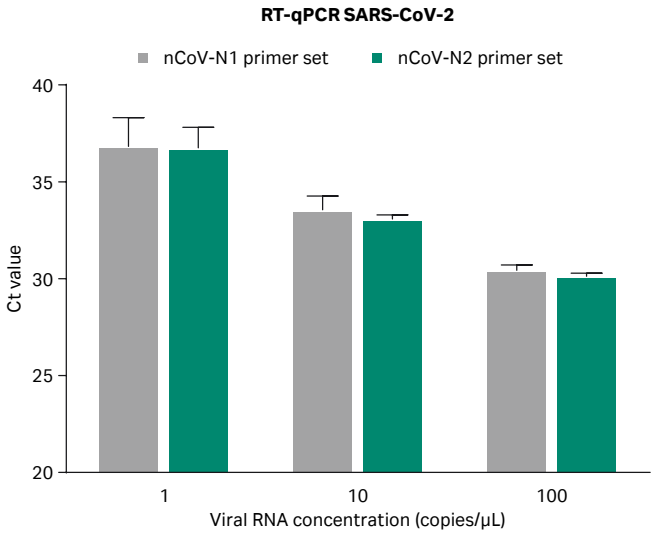

Fig 4. Ct values obtained for two SARS-CoV-2 specific primer sets with varying amounts of viral synthetic RNA in the input sample as described on the graph (viral RNA isolated using the Sera-Xtracta™ Virus/Pathogen Kit). Error bars represent standard deviation. Note that no SARS-CoV-2 specific amplification was detected in any of the un-spiked control samples (Ct undetermined, data not shown).

Comparative performance of the RT-qPCR Kit

The RT-qPCR Kit is optimized for one-step RT-qPCR quantification of extremely low-copy-number RNA templates. Performance of the RT-qPCR Kit was compared with TaqPath™ 1-Step RT-qPCR Master Mix, CG (ThermoFisher Scientific, A15300) and Luna™ Universal One-Step RT-qPCR Kit (New England Biolabs, E3005).

[†]VTM produced in support of the Centers for Disease Control and Prevention (CDC) Coronavirus Disease 2019 (COVID-19) outbreak response, containing 2% FBS, 100 µg/mL Gentamicin, 0.5 µg/mL Amphotericin B in 1 × HBSS with Ca2+ and Mg2+, no phenol red, based on the following protocol https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf.

SARS-CoV-2 synthetic viral RNA covering the nucleocapsid gene of SARS-CoV-2 was spiked at between 1 and 10 000 copies/µL into nuclease free water. Un-spiked nuclease free water (no viral RNA) served as the assay control to eliminate the possibility of false positives. One-step reverse transcription and amplification reactions were carried out following the manufacturers' protocols. Briefly, samples (5 µL per well) were run in technical triplicates using CDC Novel Coronavirus (2019-n-CoV) Diagnostic Panel primers (N1 and N2) in simplex format.

Data from three independent experiments are presented in Figure 5 and confirm that all three one-step kits detect the presence of viral RNA down to 1 copy/µL in the input sample.

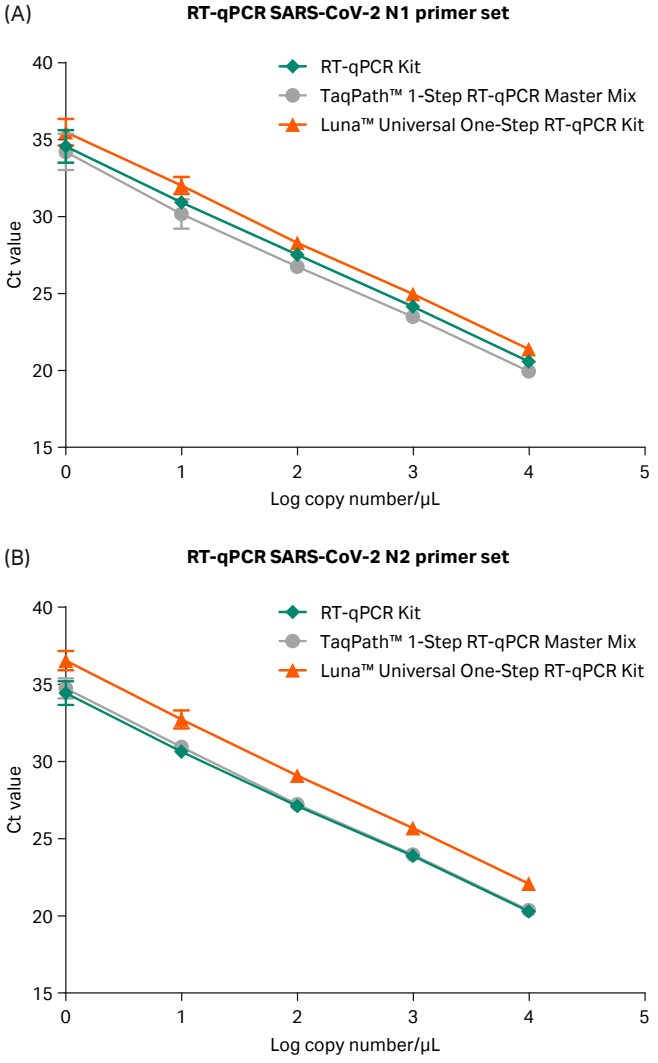

Fig 5. Ct values obtained for the RT-qPCR Kit, TaqPath™ 1-Step RT-qPCR Master Mix and Luna™ Universal One-Step RT-qPCR Kit for varying amounts of the viral synthetic RNA in the input sample as described in the graph. For clarity, Ct values obtained for each of the two SARS-CoV-2 specific primer sets (N1 and N2) have been presented in separate graphs. Values averaged from three independent experiments; error bars presented as standard deviation.

This data is based on a minimum of three independent experiments/replicate trials with an equal number of replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocol and recommendations. Data was collected at Cytiva, Maynard Centre, Cardiff, UK (R&D Laboratory) during February 2021 and is held at this location.

Multiplexing

The RT-qPCR Kit is compatible with multiplexing reactions, allowing simultaneous detection of exogenous or endogenous targets or controls for quality and increased efficiency.

Performance of the kit was assessed over five orders of magnitude using heat inactivated SARS-CoV-2 viral particles spiked at between 10 and 10 000 copies/µL into nuclease free water. In addition, RNA extracted from non-replicative recombinant Sinbis virus containing sequences from the human RNase P gene (Accuplex™ SARS-CoV-2 negative reference material, SeraCare™, 0505-0123) was spiked into the same solution at 20 copies/µL.

Samples were subjected to RT-qPCR as described previously using CDC Novel Coronavirus (2019-n-CoV) Diagnostic Panel primers (N1 and N2 targeting two regions of SARS-CoV-2 nucleocapsid gene and RNase P primers targeting human RNase P gene) in triplex format, using TaqMan™ probes labeled with FAM, TAMRA, and Cy™5 reporter dyes respectively to provide detection of three individual targets in a single reaction (2).

The RT-qPCR Kit allows for reproducible multiplex detection of viral RNA down to 1 copy/µL in the input sample. The results presented in Figure 6 demonstrate simultaneous linear and sensitive detection of an exogenous control (RNase P) and two viral RNA targets (N1 and N2) across a wide range of viral loads.

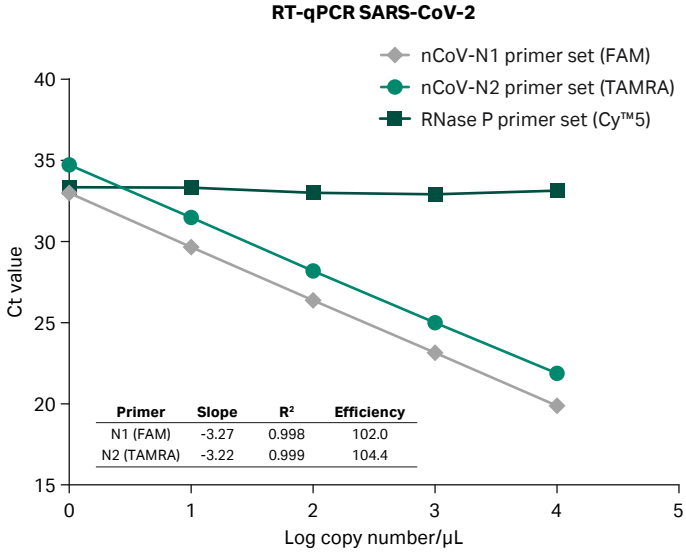

Fig 6. Ct values obtained for a triplex reaction using RNase P and two SARS-CoV-2 specific primer sets with varying amounts of viral synthetic RNA in the input sample as described on the graph. Values averaged from ten technical replicates; error bars presented as standard deviation.

Reproducible, sensitive detection of low copy inputs of viral RNA

Consistency of Ct values for three unique lots of RT-qPCR Master Mix

The RT-qPCR Kit helps generate significant and reproducible Ct values for detection of ≤ 10 copy inputs of target RNA. We carried out experiments to test RT-qPCR amplification of synthetic viral RNA covering the nucleocapsid gene of SARS-CoV-2 to assess performance and lot-to-lot variability of the RT-qPCR Master Mix.

Briefly, we ran SARS-CoV-2 viral RNA samples (5 µL per reaction) at 1 and 10 copies/µL in technical replicates (n = 10) using three unique lots of the RT-qPCR Kit and CDC 2019 Novel Coronavirus (2019-nCoV) Diagnostic Panel primers (N1, N2, and N3 targeting three regions of SARS-CoV-2 nucleocapsid gene). As per CDC guidelines, only samples for which all three markers (N1, N2, and N3) crossed the threshold at Ct < 40 were considered positive. No amplification in any of the SARS-CoV-2 specific primer sets was observed in un-spiked controls (data not shown). Note that N3 primer set is not part of CDC diagnostic panel but included in research use only (RUO).

Data presented in Figure 7 demonstrates that three distinct lots of the RT-qPCR Master Mix provide confident, reproducible detection of target RNA present at ≤ 10 copies/µL in the input sample.

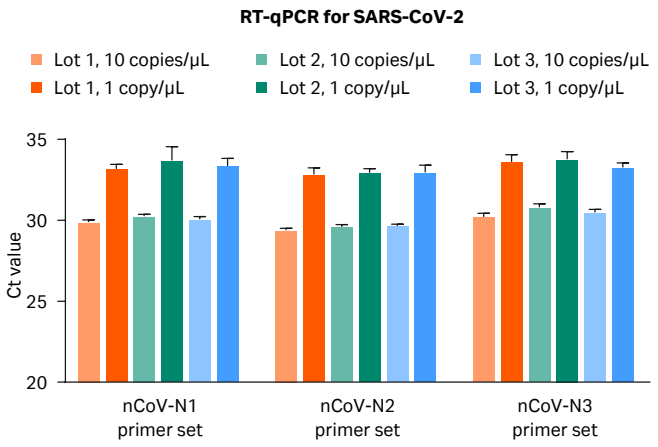

Fig 7. Ct values obtained for RT-qPCR Kit for varying amounts of viral synthetic RNA in the input sample, amplified using three unique lots of RT-qPCR Master Mix as described in the graph. Values averaged from 10 technical replicates; error bars presented as standard deviation.

Conclusions

The RT-qPCR Kit is an optimized one-step RT-qPCR master mix which provides high specificity and sensitivity to detect extremely low-copy-number targets with reproducible Ct values in one-step RT-qPCR, allowing robust detection of the viral load down to 1 copy/µL in the input sample. The proprietary master mix features a wide dynamic range that demonstrates excellent PCR linearity across an input range of five orders of magnitude. It is compatible with multiplexing applications, permitting simultaneous detection of multiple targets.

The RT-qPCR Kit is provided as a stable enzyme and mix for convenience with ambient temperature shipment and storage. The kit demonstrates equivalent performance (from RNA templates including viral RNA) when benchmarked against similar competitor master mixes and delivers fast and efficient multiplex real-time RT-PCR on both standard cyclers and fast qPCR platforms.

References

1. Centers for Disease Control and Prevention. Preparation of Viral Transport Medium, SOP# DSR-052-05. https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf
2. Kudo E, Israelow B, Vogels CBF, *et al*. Detection of SARS-CoV-2 RNA by multiplex RT-qPCR. *PLoS Biol.* 2020;18(10):e3000867. Published 2020 Oct 7. doi:10.1371/journal.pbio.3000867

Ordering information

Product	Pack size	Product code
RT-qPCR Kit	100 reactions	29639678

Related products	Pack size	Product code
AIC Mix	100 reactions	29639678
Direct RT-qPCR Kit	100 reactions	29656615
Sera-Xtracta™ Virus/Pathogen Kit	96 extractions 1000 extractions	29506009 29514201
Sera-Xtracta™ Cell-Free DNA Kit	96 purifications (2 mL input)	29437807
Sera-Xtracta™ Genomic DNA Kit	96 purifications	29429140
RNAspin Mini Kit	20 preps 50 preps 250 preps	25050070 25050071 25050072
RNAspin 96 Kit	4 × 96 preps	25050075
Sera-Mag™ Select	5 mL 60 mL 450 mL	29343045 29343052 29343057
PuRe Taq Ready-To-Go™ PCR beads	Multiwell plate, 96 reactions Multiwell plate, 5 × 96 reactions 0.5 mL tubes, 100 reactions 0.2 mL hinged tube with cap, 96 reactions	27955701 27955702 27955801 27955901
GenomiPhi™ V2 DNA amplification Kit	100 reactions 500 reactions	25660031 25660032
GenomiPhi™ V3 Ready to Go™ DNA amplification Kit	10 purifications 100 purifications 200 purifications	28903466 28903470 28903471
GFX™ 96 PCR Purification Kit	96 purifications	28903445
Blood genomicPrep Mini Spin Kit	10 purifications 50 purifications 250 purifications	28904263 28904264 28904265
Tissue and Cells genomicPrep Mini Spin Kit	50 purifications 250 purifications	28904275 28904276
MagRack Maxi	15 mL/50 mL tubes	28986441
MagRack 6	1.5 mL/2.0 mL microtubes	28948964

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