Sample prep

Efficient detection of SARS-CoV-2 from raw saliva and NPVTM using the PureLink Viral RNA/DNA Mini Kit

Summary

- The PureLink Viral RNA/DNA Mini Kit is efficient and reliable in extracting highly pure viral nucleic acid for multiple downstream applications.
- The PureLink kit is easy to use and produces fast and reproducible results.
- The PureLink kit provides a sensitive and simple method for extracting SARS-CoV-2 nucleic acid from at least 250 genomic copy equivalents per mL (GCE/mL).

Introduction

Saliva—new sample type for SARS-CoV-2 testing

With the rise of SARS-CoV-2 mutant strains, there is an increased demand for rapid and efficient viral nucleic acid extraction kits for SARS-CoV-2 detection. To meet this demand, noninvasive specimens such as saliva and buccal swabs have emerged as ideal sample types for collection. Not only is saliva one of the easiest samples to collect and store, but its shipping and storage options are also more economical in comparison to other sample types such as blood, plasma, and serum [1].

Leading the market in silica-based spin column technology, the Invitrogen™ PureLink™ Viral RNA/DNA Mini Kit (Cat. No. 12280050) is commonly used for the purification of viral nucleic acids from cell-free samples such as plasma, serum, cerebrospinal fluid, and cell culture supernatants. Saliva is one of the easiest samples to collect and store, and is significantly less invasive than a nasopharyngeal swab [1]. However, there is still uncertainty about the reliability of raw saliva when looking at low concentrations of the virus [2]. The efficiency of the PureLink Viral RNA/DNA Mini Kit in isolating spiked-in inactivated SARS-CoV-2 virus of at least 250 GCE/mL from raw saliva and nasopharyngeal viral transfer medium (NPVTM) was evaluated.

Materials and methods

Samples, controls, and nucleic acid extraction

The efficiency of viral nucleic extraction was determined using an inactivated SARS-CoV-2 control (isolate USA-WA1/2020, gamma-irradiated; BEI Resources, Cat. No. NR-52287), spiked into pooled raw saliva or NPVTM to concentrations of 250, 500, and 750 GCE/mL. Viral RNA was extracted from 10 replicates of 200 µL aliquots each of pooled raw saliva or pooled NPVTM using the PureLink Viral RNA/DNA Mini Kit. Samples were processed following the standard protocol for nucleic acid extraction for the PureLink Viral RNA/DNA Mini Kit. Samples were eluted in 50 µL.

Viral detection by PCR

Extraction efficiency was evaluated using quantitative real-time PCR (qPCR). The assays were performed on 14 µL of each extracted sample in duplicate in a 384-well plate at a final volume of 20 µL, using the Applied Biosystems™ TaqMan® SARS-CoV-2 with RNase P Assay 2.0 (Cat. No. A51121) and the Applied Biosystems™ TaqPath™ 1-Step Multiplex Master Mix (No ROX; Cat. No. A28523) on the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System (384-well block). The cycling conditions used were: 1 cycle at 25°C for 2 min, 1 cycle at 53°C for 10 min, 1 cycle at 85°C for 10 min, 1 cycle at 95°C for 2 min, 40 cycles of (95°C for 3 sec, then 65°C for 30 sec). The optical filter settings were selected following the manual instructions. The data were analyzed for SARS-CoV-2 targets by setting the threshold at 40,000. Auto-baseline settings with a start cycle of 5, were used for analysis of all targets on the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System.

Results

The PureLink Viral RNA/DNA Mini Kit was successfully used to extract viral nucleic acid from raw saliva samples and NPVTM containing at least 250 GCE/mL (50 copies per 200 µL of extracted sample) of inactivated SARS-CoV-2 (Figures 1 and 2). A kit from supplier Q was also successful in extracting nucleic acid from NPVTM (Figure 3). The three specific SARS-CoV-2 target sequences (*orf1a*, *orf1b*, and N gene) show similar results for both sample types. Consistent results were achieved using both sample types, demonstrating the reliability of the PureLink Viral RNA/DNA Mini Kit in isolating SARS-CoV-2 viral nucleic acid. The PureLink kit showed sensitivity and performance equivalent to that of supplier Q's viral extraction kit.

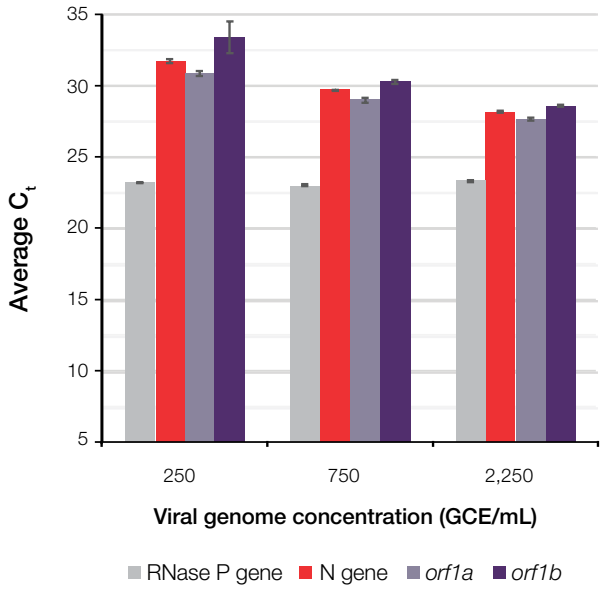

Figure 1. qPCR results of raw saliva samples with spiked-in SARS-CoV-2 extracted using the PureLink Viral RNA/DNA Mini Kit. The data show average C_t values of SARS-CoV-2 targets at various concentrations.

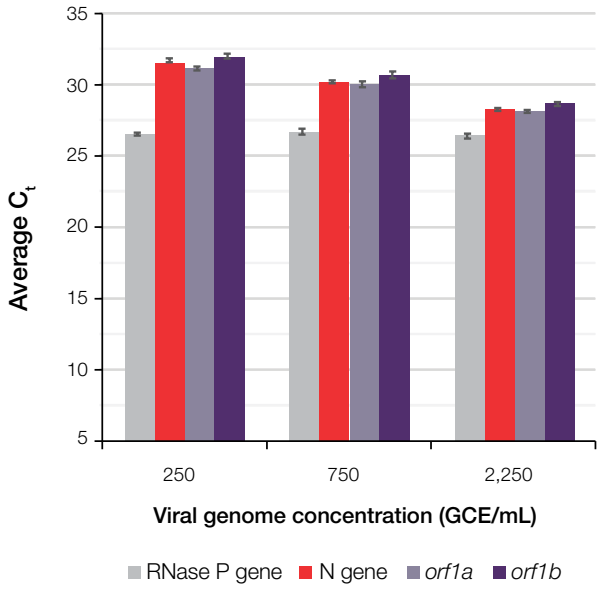

Figure 2. qPCR results of NPVTM samples with spiked-in SARS-CoV-2 extracted using the PureLink Viral RNA/DNA Mini Kit. The data show average C_t values of SARS-CoV-2 targets at various concentrations.

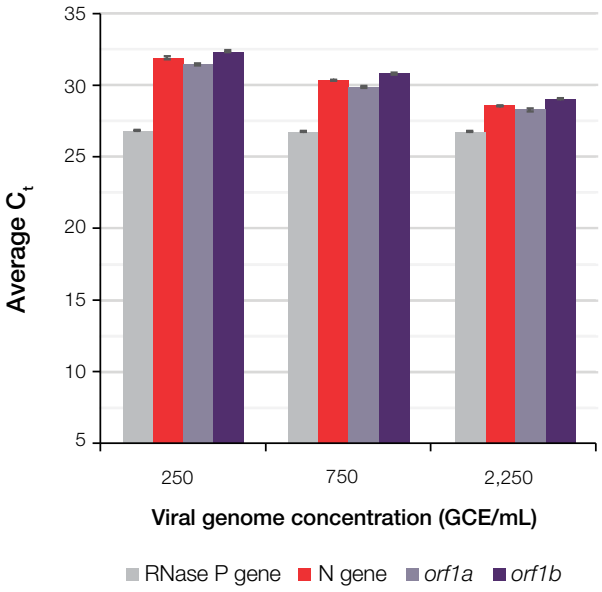

Figure 3. qPCR results of NPVTM samples with spiked-in SARS-CoV-2 extracted using a kit from supplier Q. The data show average C_t values of SARS-CoV-2 targets at various concentrations.

Conclusion

The PureLink Viral RNA/DNA Mini Kit provides a simple and reliable method for nucleic acid extraction from raw saliva samples and NPVTM. The efficiency of nucleic acid extraction was tested with samples spiked with inactivated SARS-CoV-2 at various concentrations, and the results from using the PureLink kit were compared to those of another supplier's kit (supplier Q). The PureLink kit is an affordable and easy-to-use kit for isolating viral nucleic acid for low-throughput workflows.

References

1. Tan SH, Allicock O, Armstrong-Hough M et al. (2021) Saliva as a gold-standard sample for SARS-CoV-2 detection. *The Lancet Respiratory Medicine* doi:10.1016/S2213-2600(21)00178-8.
2. Butler-Laporte G et al. (2021) Comparison of saliva and nasopharyngeal swab nucleic acid amplification testing for detection of SARS-CoV-2: a systematic review and meta-analysis. *JAMA Internal Medicine* doi:10.1001/jamainternmed.2020.8876.

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