Sample prep

# Detection of viral and bacterial nucleic acid from plasma, viral transport media, and saliva using the PureLink Viral RNA/DNA Mini Kit

**Keywords**
SARS-CoV-2, saliva, PureLink, Invitrogen, SpeciMAX

## Summary

- The PureLink Viral RNA/DNA Mini Kit is efficient and reliable in extracting high-quality viral and bacterial nucleic acid from various sample matrices.
- The PureLink kit provides a simple, low-throughput method for extracting viruses such as coronaviruses and adenoviruses, and bacteria such as *Bordetella pertussis* and *Chlamydia pneumoniae*, at low spike-in concentrations.
- The PureLink kit is easy to use with fast and reproducible results.

## Introduction

With the increased need for reliable and easy sample collection, saliva has emerged as a preferred sample type for respiratory virus testing. To collect and stabilize saliva, the Thermo Scientific™ SpeciMAX™ Stabilized Saliva Collection Kit (**Cat No. A50697**) can be used for high-throughput human coronavirus surveillance programs.

However, there is also the need for low-throughput extraction and detection of viral and bacterial strains.

The Invitrogen™ PureLink™ Viral RNA/DNA Mini Kit (**Cat. No. 12280050**) is most commonly used to extract nucleic acids from serum, plasma, and cerebrospinal fluid. The kit is also efficient in extracting viral RNA from nasopharyngeal swabs (NP swabs) in viral transport medium (VTM) and raw saliva.

The efficiency and versatility of the PureLink Viral RNA/DNA Mini Kit in isolating viral and bacterial nucleic acids from a variety of sample types is demonstrated here.

## Materials and methods

### Samples, controls, and nucleic acid extraction

Using the PureLink Viral RNA/DNA Mini Kit, nucleic acid was extracted from 200 µL of plasma, viral transport medium (VTM), raw saliva, and saliva stabilized using the SpeciMAX kit. Each sample was spiked with 1 µL each of NATtrol™ RP Multimarker 1 and RP Multimarker 2 controls (ZeptoMetrix). NATtrol RP Multimarker 1 contains inactivated influenza A-H1N1 and H3N2, rhinovirus type 1A, adenovirus type 3, *Chlamydia pneumoniae* CWL-029, and *Mycoplasma pneumoniae* M129. NATtrol RP Multimarker 2 contains inactivated coronavirus OC43, coronavirus NL63, coronavirus 229E, and *Bordetella pertussis* A639. Viral and bacterial RNA were extracted from the sample types using the standard protocol for the PureLink Viral RNA/DNA Mini Kit. Duplicate samples of plasma and VTM and 11 individuals' saliva were used. Samples were eluted in 50 µL of nuclease-free water.

### Viral and bacterial detection by PCR

Extraction efficiency was evaluated using quantitative real-time PCR (qPCR). The 3 coronavirus assays used 2.5 µL of sample in duplicate reactions, and 1.2 µL of sample was used in duplicate for the influenza A, rhinovirus, and adenovirus assays, with the Applied Biosystems™ TaqMan® Fast Virus 1-Step Master Mix (Cat No. 4444436) on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System. The final volume of the qPCR reactions was 10 µL, and the cycling conditions used were: 1 cycle at 50°C for 5 min, 1 cycle at 95°C for 20 sec, 40 cycles of (95°C for 3 sec, then 60°C for 30 sec).

No replicates were omitted; automatic threshold and baseline settings were used.

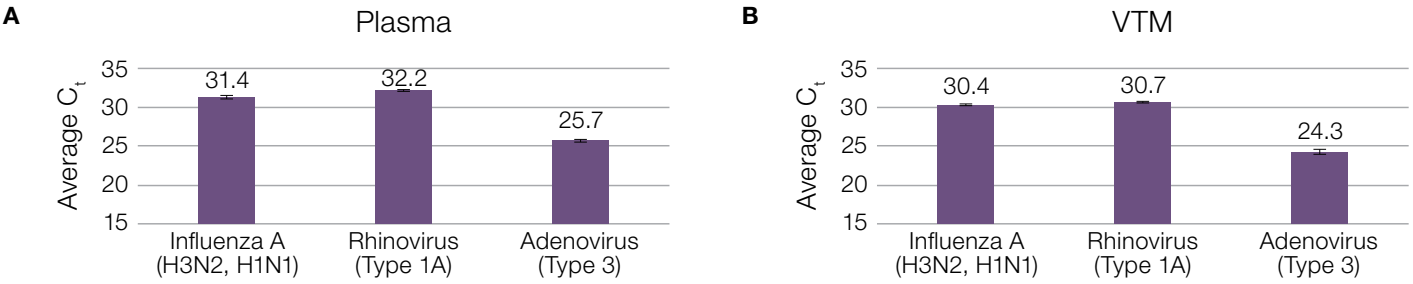


**Figure 1. qPCR results of plasma and VTM samples.** Average $C_t$ values for detecting influenza A (H3N2 and H1N1), rhinovirus (type 1A), and adenovirus (type 3) in **(A)** plasma, and **(B)** VTM samples, each spiked with NATtrol RP Multimarker 1 control.

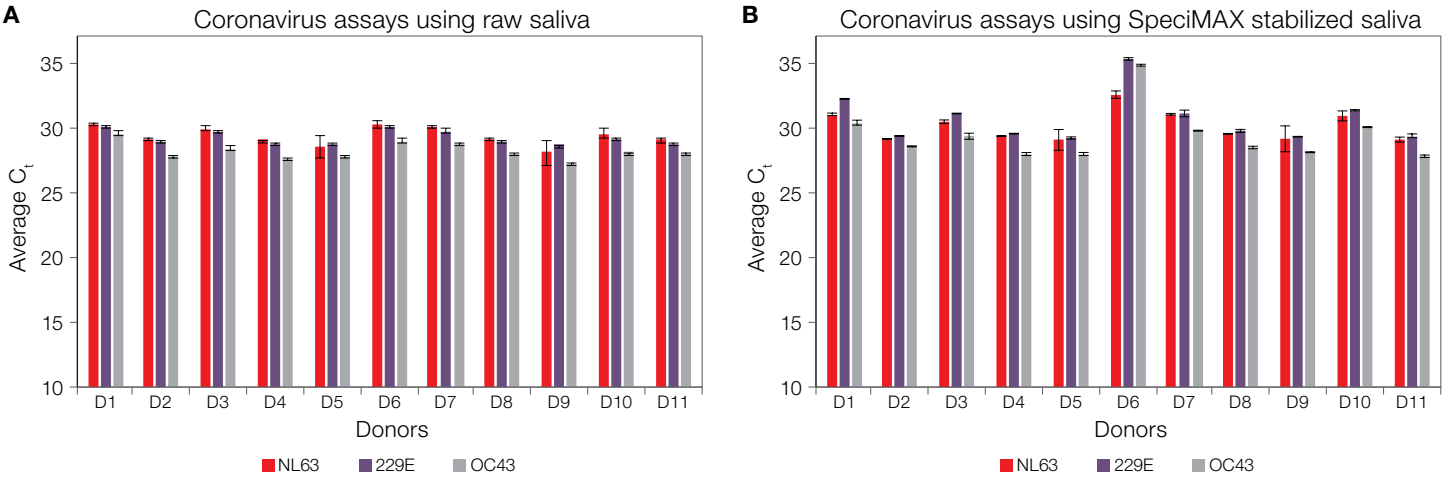


**Figure 2. qPCR results of raw saliva samples and saliva samples stabilized using the SpeciMAX kit. (A)** Average $C_t$ values for detecting coronaviruses NL63, 229E, and OC43 in **(A)** raw saliva, and **(B)** saliva stabilized using the SpeciMAX kit, each spiked with NATtrol RP Multimarker 2 control.

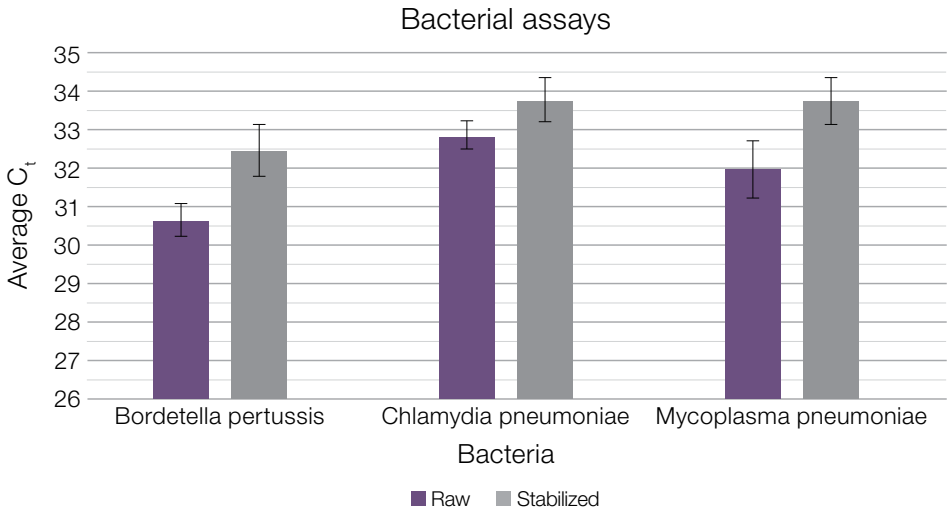


**Figure 3. Average $C_t$ values of spiked-in RP Multimarker 1 and 2 controls using raw saliva and saliva stabilized using the SpeciMAX kit.** Data from 11 donors were averaged.

## Results

The PureLink Viral RNA/DNA Mini Kit can successfully detect a spiked low input of the following viruses: influenza A (H3N2 and H1N1), rhinovirus (type 1A), and adenovirus (type 3) in plasma ($C_t$ values: 31.4, 32.2, and 25.7, respectively) and in VTM ($C_t$ values: 30.4, 30.7, and 24.3, respectively) (Figure 1). The PureLink kit can successfully detect human coronaviruses (NL63, OC43, and 229E) in raw saliva and saliva stabilized using the SpeciMAX kit (Figure 2). Saliva is variable from donor to donor, even if the protocol is followed for sample collection. Even though the PureLink Viral RNA/DNA Mini Kit is optimized for viral nucleic acid, it also performs well with gram-negative bacteria (Figure 3). For the bacterial assays, saliva stabilized using the SpeciMAX kit is spiked with half the amount used for raw saliva. The lower copy number of spike-in control contributes to the higher $C_t$ value observed with the stabilized saliva samples.

## Conclusion

The PureLink Viral RNA/DNA Mini Kit has demonstrated versatility for extracting nucleic acid from raw saliva, saliva stabilized using the SpeciMAX kit, plasma, and VTM for viral nucleic extraction. The PureLink Viral RNA/DNA Mini Kit is also efficient for extracting bacterial RNA in addition to multiple respiratory viruses. The PureLink kit provides a simple, lower-throughput method for both viral and bacterial extractions from a variety of sample matrices.

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