# Using Norgen's Saliva/Swab RNA Purification Kit on the Hamilton Vantage to Isolate RNA from Fresh and Preserved Saliva Samples without Contamination

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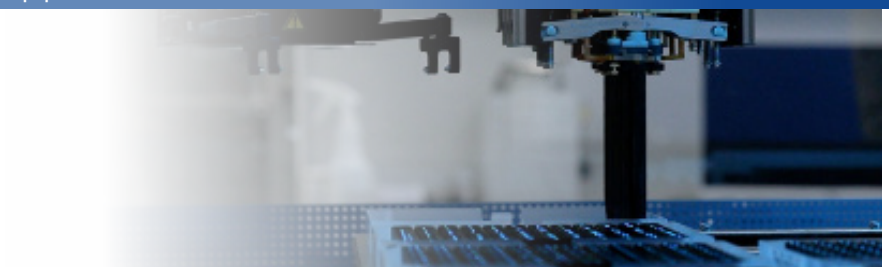
Application Note 98



Keywords

+ Virus
+ RNA
+ Extraction
+ Saliva
+ Swab
+ SARS-CoV-2
+ COVID-19
+ RT-qPCR
+ E/RdRP genes
+ Diagnostics
+ Method
+ Automation
+ No Contamination

## INTRODUCTION

Saliva samples play a key role in many applications – including diagnostic testing, transcriptomics research, and biomarker discovery. Saliva samples can be a great non-invasive alternative to other liquid biopsy options. **Norgen Biotek's Saliva/Swab Purification 96-Well Kit (Cat. 69300)** uses silicon carbide technology to capture total RNA, including fragmented and microRNA. This non-biased isolation can be very important when working with diagnostic samples, when low viral loads can lead to false negative samples if any degradation has occurred.

Automation is essential for processing large numbers of samples for these applications. However, many automated RNA isolation protocols have increased risk of contamination compared with manual isolation techniques. Particularly in diagnostics, optimizing methods for high-throughput volumes often requires pooling samples for isolation. This increases the importance of eliminating any risk of contamination, as every contaminated pool of samples could lead to an unnecessary re-isolation of dozens of samples.

In this experiment RNA was isolated from both fresh and preserved saliva samples and potential contamination was determined through RT-qPCR.

## MATERIALS & METHODS

24 mL of saliva was collected and pooled, then aliquoted into 16 x 1 mL samples and 16 x 0.5 mL samples. To each 0.5 mL sample 0.5 mL Norgen Saliva RNA Preservative was added and inverted to mix. 8 samples of preserved saliva and 8 samples of fresh saliva were spiked with 6 x $10^7$ copies of synthetic RNA transcript and arranged in the pattern shown in Figure 1.

The samples were processed using the Hamilton Vantage according to the Norgen Saliva-Swab RNA isolation script for Vantage. Duplicate

A

| | Fresh Saliva | | Pres. Saliva | |
|---|---|---|---|---|
| A | + | - | + | - |
| B | - | + | - | + |
| C | + | - | + | - |
| D | - | + | - | + |
| E | + | - | + | - |
| F | - | + | - | + |
| G | + | - | + | - |
| H | - | + | - | + |

**Figure 1: Layout of Spiked & Negative Saliva Samples** Every other sample of fresh and preserved saliva was spiked with 60 000 000 copies of a synthetic RNA transcript. Spiked samples are represented by a red + and fill, non-spiked samples are represented by a green – and fill.

RT-qPCR reactions were run for each sample, using 5 µL of eluate per reaction. All RT-qPCR reactions were run on the Thermo Fisher QuantStudio 7Pro using **Norgen 2X One-Step RT-PCR Master Mix (Cat. 28113)** and proprietary primer/probe solution.

## RESULTS & DISCUSSION

**RT-qPCR Assay**

All spiked samples showed amplification of the target with Cq <30 (Figure 2). Non-spiked samples did not show any amplification of the target RNA transcript. The amplification of spiked samples is shown in Figure 3. The average Cq value across all samples was found to be 26.60, with a standard deviation of 1.39 (Figure 4).

## CONCLUSION

The layout of the spiked and negative samples was designed to clearly indicate any contamination through the proximity of positive and negative samples. Due to the high concentration of RNA in the spiked samples, even very small amounts of cross-contamination should appear using RT-qPCR. The absence of any amplification indicates that absolutely no contamination has occurred.

Successful isolation of all 32 samples show that **Norgen's Saliva/ Swab RNA Purification 96-Well Kit (Cat. 69300)** on the Hamilton Vantage can be used without risk of contamination.

B

| | Fresh Saliva | | Pres. Saliva | |
|---|---|---|---|---|
| A | 24.89 | N/A | 25.51 | N/A |
| B | N/A | 26.92 | N/A | 27.44 |
| C | 26.87 | N/A | 25.92 | N/A |
| D | N/A | 26.74 | N/A | 27.78 |
| E | 25.78 | N/A | 29.99 | N/A |
| F | N/A | 26.73 | N/A | 27.41 |
| G | 24.22 | N/A | 27.28 | N/A |
| H | N/A | 24.76 | N/A | 27.28 |

**Figure 2: Average Cq values of samples** The RNA isolated from all spiked samples amplified with Cq values <30, no amplification was observed in any negative samples.

C

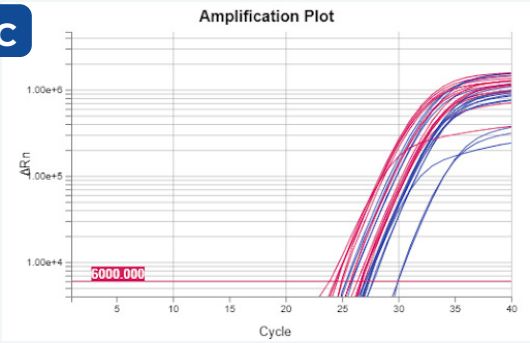



**Figure 3:** Amplification Plot of Spiked Samples

| Related Products | Research Use | CE Marked |
|---|---|---|
| Saliva/Swab RNA Purification 96-Well Kit | 69300 | Dx69300 |
| Norgen 2X One-Step RT-PCR Master Mix | 28113 | N/A |

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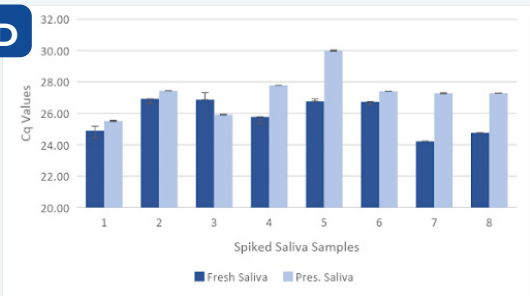


**Figure 4: Average Cq Values of spiked fresh and preserved saliva samples** The average Cq of all spiked samples was found to be 26.60, with a standard deviation of 1.39.