# High throughput viral RNA extraction from throat swabs.

Application Note

## AUTOMATED RNA EXTRACTION USING THE ZYMO RESEARCH *QUICK*-DNA/RNA™ VIRAL MAGBEAD KIT ON THE FLUENT® WORKSTATION

TECAN.

## INTRODUCTION

The need for a consistent and sensitive methodology for high recovery of viral DNA and RNA expands beyond SARS-CoV-2 testing workflows. It is essential to be able to reliably identify low amounts of viral nucleic acids present in a sample, for early detection of viral infections with high sensitivity downstream applications.

The Zymo Research *Quick*-DNA/RNA Viral MagBead kit was specifically developed to effectively lyse viral particles and bind nucleic acids with high efficiency. Its robust buffer system is compatible with a wide array of sample types including swabs, saliva, plasma, serum, blood and other common biological samples, as well as cellular suspensions or cell culture media.

The magnetic bead-based system allows the *Quick*-DNA/RNA Viral MagBead Kit to be used on Tecan Fluent® instruments. Rapid extraction of the viral nucleic acids can be performed on Fluent 780 and 1080 workstations, using the MultiChannel Arm™ (MCA) 96 head for a high throughput workflow.

## MATERIALS AND METHODS

The DreamPrep® NAP workstation has been scaled up from the Fluent 480 base and implemented on the Fluent 780/1080 to enable the use of the 96-channel MCA head pipetting technology. The extraction workflow was performed using a Fluent 1080 Automation Workstation in combination with FluentControl™ GX Assurance Software. The instrument was equipped with an Air Flexible Channel Arm™ (Air FCA), an MCA 96 with the Extended Volume Adapter (EVA), a Robotic Gripper Arm™ (RGA), Fluent ID™ and a handheld barcode scanner (Honeywell) for sample and reagent barcode recording, a BioShake™ D30-T elm (QInstruments) for heating and shaking, and a Magnum FLX® Enhanced Universal Magnet Plate (Alpaqua) (Figure 1). Filtered 200 and 500 µl MCA 96 tips, and 200 and 1,000 µl filtered Liquid Handling (LiHa) disposable tips were used for all experiments.

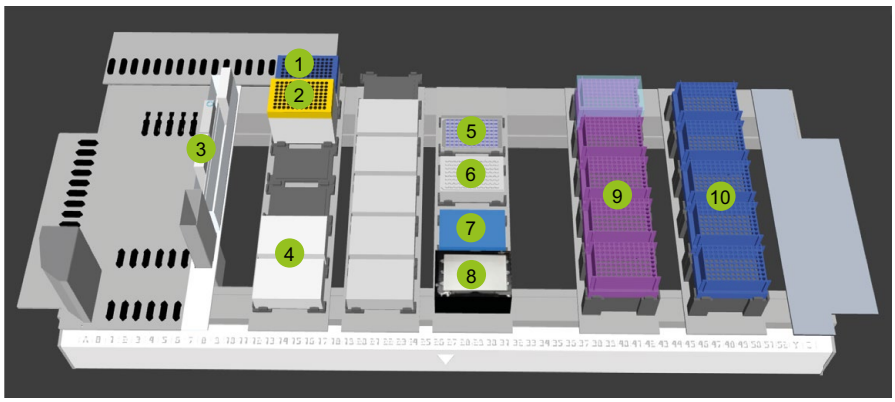
Viral DNA and RNA were extracted and purified using the Zymo Research *Quick*-DNA/RNA MagBead Kit (#R2141) on the Fluent 1080 instrument. Purified nucleic acids were analyzed with the Zymo Research *Quick* SARS-CoV-2 Multiplex Kit (#R3013) on a Bio-Rad CFX96 Touch Real-Time PCR Detection System. Heat-inactivated SARS-CoV-2 virus was spiked into throat swabs collected in DNA/RNA Shield™ Collection Tubes with Swab (#R1108/R1109, Zymo Research). A NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific) was used to determine the concentration and purity of the eluted viral DNA and RNA.

Up to 300 µl of sample in DNA/RNA Shield was added to a 2 ml Nunc™ DeepWell™ Plate and loaded onto the Fluent for processing. The automated procedure performs a proteinase K digestion, then adds the specialized Viral DNA/RNA Buffer and MagBinding Beads to bind the viral DNA/RNA. The Fluent subsequently carries out a series of wash steps designed to remove contaminants, salts and proteins, before the purified viral DNA/RNA is eluted and transferred to a PCR plate.

## RESULTS AND DATA ANALYSIS

### rRT-PCR analysis of SARS-CoV-2 viral RNA recovered from DNA/RNA Shield Collection Tubes

Throat swabs collected in DNA/RNA Shield were spiked with heat-inactivated SARS-CoV-2 virus, in a dilution series ranging from 0.75 to 75 genetic equivalent copies (GEC) per microliter (1:10 dilutions, n = 8). Samples were processed with the *Quick*-DNA/RNA Viral MagBead Kit on the Fluent 1080 and the results were analyzed using the *Quick* SARS-CoV-2 Multiplex Kit.




1 = 200 µl SLAS format, filtered disposable tips
2 = 1,000 µl SLAS format, filtered disposable tips
3 = 25 ml reagent troughs
4 = Liquid waste trough
5 = 96-well standard microplate
6 = Nunc 96-well DeepWell plate
7 = Magnum FLX plate
8 = BioShake D30-T elm
9 = 500 µl MCA filtered disposable tips
10 = 200 µl MCA filtered disposable tips

Figure 1: Configuration of the Fluent 1080 for the *Quick*-DNA/RNA Viral MagBead workflow.

Based on these results, the automated workflow can consistently detect SARS-CoV-2 viral RNA with high sensitivity. The average detection threshold values of the dilution series show a strong linear correlation ($R^2$ = 0.9999) (Figure 2). This proves that the isolation and purification protocol on the Tecan Fluent instrument can dependably extract viral RNA from samples with a range of viral copy loads.

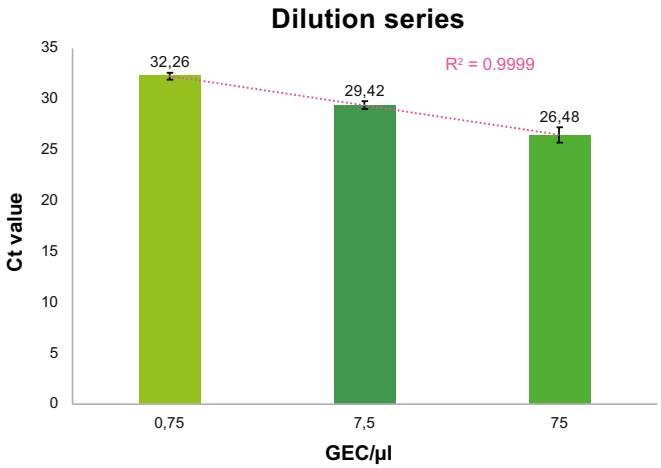



Figure 2: Detection threshold (Ct) values for samples spiked with heat-inactivated SARS-CoV-2 virus in dilution series (1:10 dilutions, n = 8, 32, 8).

### Limit of detection

Spiked samples were loaded in a checkerboard pattern with negative controls in the alternating wells. The full plate was extracted using the *Quick*-DNA/RNA Viral MagBead Kit on the Fluent 1080 and analyzed with the *Quick* SARS-CoV-2 Multiplex Kit on the Bio-Rad CFX96 Touch Real-Time PCR Detection System. The extremely sensitive *Quick* SARS-CoV-2 Multiplex Kit is a qualitative assay with 100 % viral detection at 10 GEC per reaction.

The average detection threshold values and standard deviations are listed in Table 1. The results prove the compatibility of the extraction workflow on the Tecan Fluent with rRT-PCR analysis for viral RNA detection (Figure 3).

| Viral copy load (GEC/µl) | Average Ct value | Standard deviation (%) |
|---|---|---|
| 75 | 26.48 | 0.8 |
| 7.5 | 29.42 | 0.4 |
| 0.75 | 32.26 | 0.3 |

Table 1: Detection threshold values for samples extracted using the *Quick*-DNA/RNA Viral MagBead Kit on the Fluent 1080 workstation

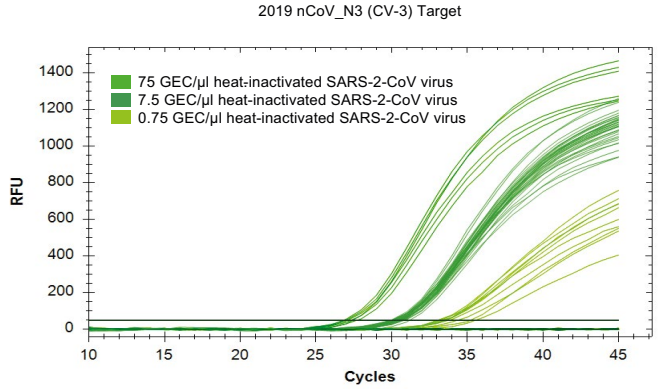
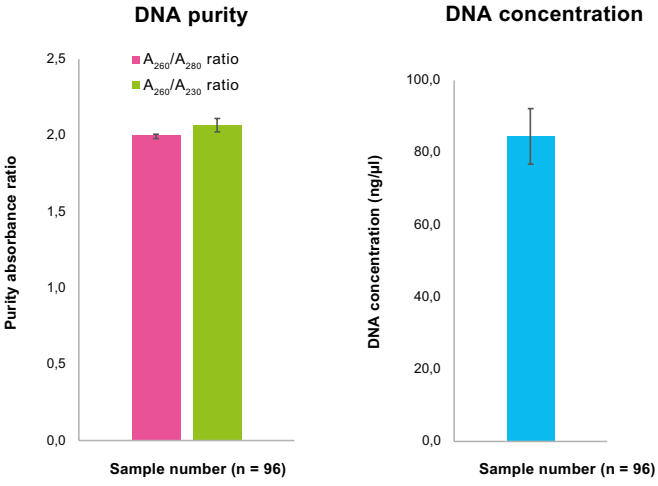



Figure 3: rRT-PCR amplification graphs of viral RNA from throat swabs in DNA/RNA Shield spiked with heat-inactivated SARS-CoV-2 virus. Samples were analyzed with the *Quick* SARS-CoV-2 Multiplex Kit with 2019 CoV-N3(CV-3) target primer.

### Consistency of yield and purity

To verify the consistency of yield and purity of the extraction protocol for the automated *Quick*-DNA/RNA Viral MagBead Kit workflow, a HeLa cell pellet with well-known recovery and characteristics was used as a control. A full 96-well plate of HeLa cell pellets was processed and analyzed. Each pellet contained approximately 150,000 cells and was resuspended in DNA/RNA Shield.

The resulting total DNA and RNA were quantified using the NanoDrop 2000 to determine concentration and purity. The average DNA concentration was 84.5 ng/µl with a standard deviation of 7.7, which matches the expected yield and technical specifications of the *Quick*-DNA/RNA Viral MagBead Kit (Figure 4). The average purity ratios for $A_{260}/A_{280}$ and $A_{260}/A_{230}$ were 2.0 and 2.1, respectively, with a standard deviation below 0.1 (Figure 5). These results further support the consistency and reproducibility of the automated extraction protocol.




Figures 4 and 5: Concentration and purity ratios for extracted viral DNA.

### Cross-contamination

Samples spiked with heat-inactivated SARS-CoV-2 and negative control samples were arranged in a checkerboard pattern, as previously described, to test for cross-contamination. After extraction, eluates were analyzed with the *Quick* SARS-CoV-2 Multiplex Kit using primer 2019 nCoV_N3 (CV-3) targeting the virus nucleocapsid gene N3. No amplification was shown in the no template controls (NTC), indicating that no cross-contamination occurred in the workflow (Figure 3 and Figure 6).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 29.08 | NTC | 29.11 | NTC | 29.20 | NTC | 32.01 | NTC | 29.27 | NTC | 26.01 | NTC |
| B | NTC | 29.36 | NTC | 29.57 | NTC | 29.17 | NTC | 32.14 | NTC | 28.96 | NTC | 26.52 |
| C | 28.80 | NTC | 28.97 | NTC | 29.24 | NTC | 32.02 | NTC | 29.47 | NTC | 27.81 | NTC |
| D | NTC | 29.31 | NTC | 28.97 | NTC | 29.14 | NTC | 32.18 | NTC | 29.27 | NTC | 25.85 |
| E | 29.17 | NTC | 28.38 | NTC | 28.53 | NTC | 32.29 | NTC | 29.20 | NTC | 27.33 | NTC |
| F | NTC | 29.54 | NTC | 28.92 | NTC | 28.50 | NTC | 32.23 | NTC | 29.59 | NTC | 25.61 |
| G | 28.64 | NTC | 29.10 | NTC | 29.08 | NTC | 33.06 | NTC | 29.33 | NTC | 26.58 | NTC |
| H | NTC | 29.08 | NTC | 29.17 | NTC | 29.67 | NTC | 32.12 | NTC | 30.27 | NTC | 26.14 |
| | 7.5 GEC/µl | | | | | | 0.75 GEC/µl | | 7.5 GEC/µl | | 75 GEC/µl | |

Figure 6: Threshold values for rRT-qPCR analysis of positive and negative control samples.

## SUMMARY

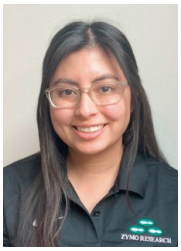
Integration of the Zymo Research *Quick*-DNA/RNA Viral MagBead Kit on the Fluent 780/1080 workstations, equipped with the Multiple Channel Arm and 96-channel head EVA adapter, offers a fully automated high throughput solution for rapid extraction of viral nucleic acids.

The data presented in this application note demonstrates that this automated walkaway workflow results in highly pure viral nucleic acids that are compatible with many sensitive downstream applications, including RT/qPCR and Next Generation Sequencing. It also shows that high quality viral DNA/RNA, such as SARS-CoV-2, is consistently recovered from samples down to 0.75 GEC/µl without any cross-contamination.

## LEARN MORE

To learn more contact your sales representative or visit **www.tecan.com/NAP**.

### About the author



***Erica Carrasco*** *is the Automation Assistant Manager at Zymo Research, with eight years of laboratory experience. She received her master's degree in chemistry at the University of San Francisco, focusing on physical and atmospheric chemistry. Erica joined Zymo Research in 2018, and since then has worked on various research and design, product development, marketing and automation projects.*

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