Application Note · CyBio FeliX Nucleic Acid Extraction

Content

Purify viral RNA purification from viral transport medium (VTM) using a fully automated protocol with the Maxwell® HT Viral TNA Kit, Custom on the CyBio FeliX liquid handler.

Automated Purification Of Viral RNA From Viral Transport Medium Using Promega Maxwell® HT Viral TNA Kit, Custom On The Analytik Jena CyBio FeliX Liquid Handler

Application Summary

Kit: Maxwell® HT Viral TNA Kit, Custom (Cat.# AX2340)
Analyses: RT-qPCR
Sample Type(s): Samples collected in viral transport medium, e.g. nasopharyngeal swabs
Input: 200 µL

Materials and Methods

Instruments and Equipment

- CyBio FeliX Basic Unit with Enclosure (Cat.# OL5015-24-100)
- CyBio FeliX Extraction Set (Cat.# OL5015-25-120)
- Protective Plate (Cat.# OL3317-25-126)
- Heat block
- Microcentrifuge

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Consumables and Reagents

- Maxwell® HT Viral TNA Kit, Custom (Cat.# AX2340)
- CyBio TipRack 96/1000 µL, PCR-certified, pre-sterilized, filter (Cat.# OL3811-25-939-F)
- Deep Well Plate, 96 square well, 2 mL (Cat.# 845-FX-8500025)
- 100% Isopropanol (molecular biology grade)
- 100% and 80% Ethanol (molecular biology grade)

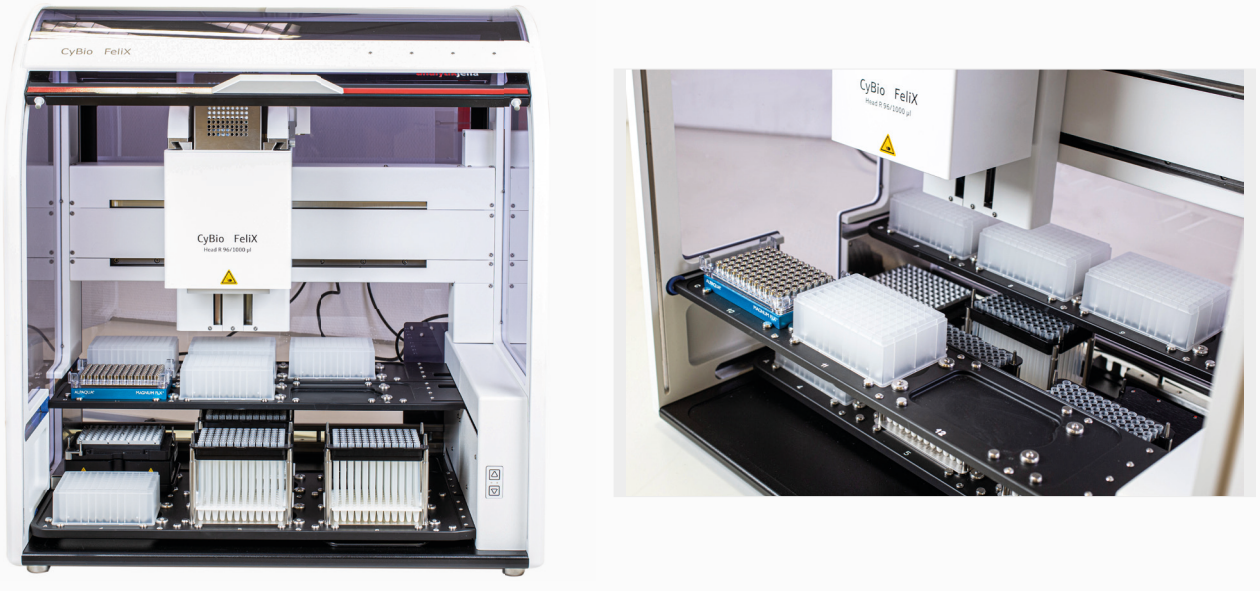
Method

1. Add Isopropanol to 4/40 Wash Solution, as indicated on the bottle.
2. Add 100% Ethanol and Isopropanol to Alcohol Wash, as indicated on the bottle.
3. Prepare 80% Ethanol.
4. Prepare a lysis/binding mastermix for all samples plus 10% overage in an appropriately sized container. For each sample, add 150 µL of Lysis Solution, 15 µL of Proteinase K, 300 µL of 100% Isopropanol, and 15 µL of MagneSil RED. Invert the mixture until the contents are homogeneous (do not vortex).
5. For each sample to be processed, add 480 µL of the lysis/binding mastermix from step 4 to wells of the 96 well processing plate.
6. Add 200 µL of sample to the 96 well plate processing plate containing the lysis/binding mastermix.
7. The method uses the following reagents and volumes per well (Table 1).
8. Start the run on the CyBio Felix Liquid Handler – Maxwell HT Viral TNA_Version1.bms.
9. Follow the instructions on the instrument for reagent setup. The instrument deck configuration is shown in Figure 1. The user will be prompted during the method start to load the sample plate containing Samples & Lysis/Bind reagents on the BioShake (position 1 on deck layout). The automated method can be configured with a variable elution volume. This Application Note was performed with 60 µL and 110 µL as elution volumes.

Table 1: Reagents and volumes required per sample.
Note: Plates labeled Wash 1, Wash 2 and Wash 3 are used as reservoirs and are filled with 100 µL of excess volume (i.e., dead volume) per well.

Plate	Well Contents	Volume of Reagent Added per Well	Volume Used per Reaction
Lysis & Bind	Sample + Lysis/ Bind Reagents	680 µL	680 µL
Wash 1	4/40 Wash Buffer	275 µL	175 µL
Wash 2	Alcohol Wash Blood	250 µL	150 µL
Wash 3	80% Ethanol	225 µL	125 µL
Elution	Nuclease-free Water	60 µL / 110 µL	60 µL / 110 µL

A



B

Figure 1: Deck layout for the Maxwell® HT Viral Total Nucleic Acid Kit on the CyBio FeliX Liquid Handler.
(A) Analytik Jena CyBio FeliX instrument with the CyBio FeliX Extraction Set, including the Alpaqua® Magnum FLX™ magnet and the Bioshake 3000-T elm mounted with an adapter for deep well plates. **(B)** Reagent and accessory positions for implementing the Maxwell® HT Viral TNA Kit, Custom.

Results

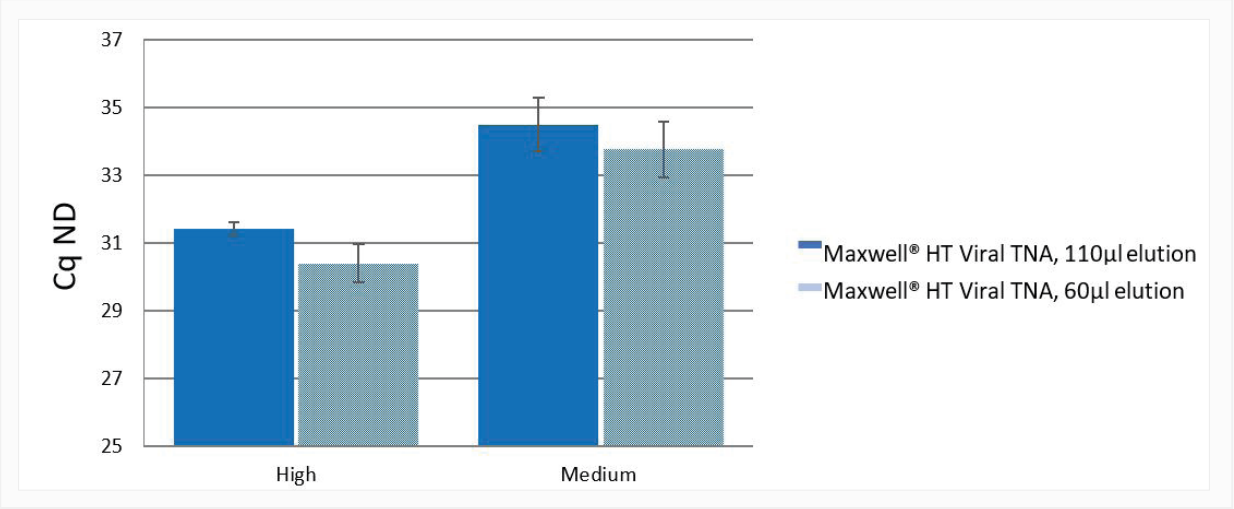


Figure 2: Detection of SARS-CoV-2 RNA Purified from VTM using the Maxwell® HT Viral TNA Kit on the CyBio FeliX Liquid Handler. Improviral™ Viral Preservative Medium (Improve, Cat.# 8110111) was spiked with SARS-CoV-2 reconstituted from Helix Elite™ Inactivated SARS-CoV-2 Standard (Microbiologics, Cat. # HE0065N) in VTM. The high virus sample contains approximately 2 x 104 copies of SARS-CoV-2 per sample. The medium virus sample is a 1:5 dilution of the high virus sample in VTM (4 x 103 copies per sample). No virus controls were run along with spiked samples. 200 µL of the spiked VTM was extracted with the Maxwell® HT Viral TNA Kit, Custom (Cat.# AX2340) on the CyBio FeliX Liquid Handler, as described above. Two elution volumes were used (110 µL and 60 µL). Samples were purified in quadruplicate (n=4). Following nucleic acid purification, presence of SARS-CoV-2 was detected by RT-qPCR using the GoTaq® Probe 1-Step qPCR System (Cat.# A6121). Each reaction contained 5 µL of eluate with 10 µL of the GoTaq® Probe qPCR Master Mix with dUTP, 0.4 µL of GoScript™ RT Mix 1-Step RT-qPCR, 4 µL of 2019-nCoV_N1 primer-probe set targeting the SARS-CoV-2 nucleocapsid, and Nuclease-free Water added to a final volume of 20 µL. 1-Step RT-qPCR thermal cycling was as follows: reverse transcription at 45°C for 10 minutes, hot-start activation at 95°C for 2 minutes, and then 45 cycles of denaturation at 95°C for 3 seconds and annealing/extension at 55°C for 30 seconds, with signal acquisition during the annealing/extension stage of cycling. Data represent the average of quadruplicate purifications amplified in duplicate with standard deviation. No template controls and no virus controls samples did not amplify.

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