Application Note · CyBio FeliX Nucleic Acid Extraction

Content

Purify viral RNA purification from viral transport medium (VTM) using a fully automated protocol with the One4All Kit, Custom on the CyBio FeliX liquid handler.

Automated Purification Of Viral RNA From Viral Transport Medium Using Promega One4All Kit, Custom On The Analytik Jena CyBio FeliX Liquid Handler

Application Summary

Kit: One4All Kit, Custom (Cat.# AX9760)
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

- One4All Kit, Custom (Cat.# AX9760)
- 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)

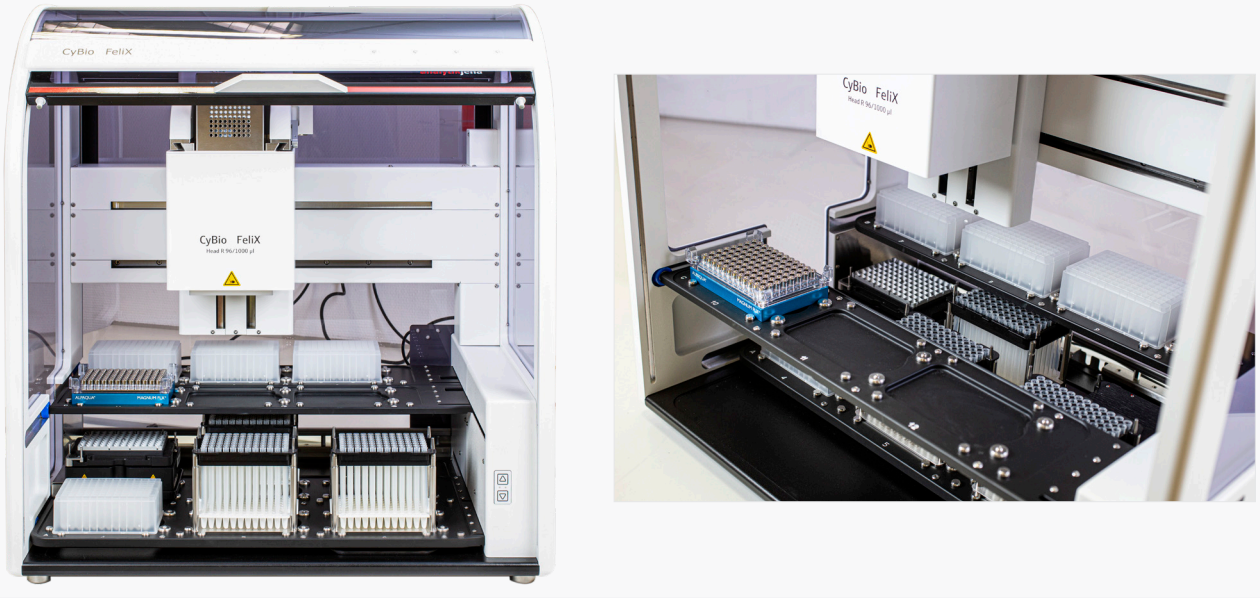
Method

1. Prepare 80% ethanol.
2. Prepare a binding mastermix for all samples plus 10% overage in an appropriately sized container. For each sample, add 350 µL of MagnaCel Binding Buffer, 10 µL of Proteinase K, and 20 µL of concentrated Maxwell® C Resin. Invert the mixture until the contents are homogeneous (do not vortex).
3. For each sample to be processed, add manually 200 µL of Lysis Solution, 200 µL of sample and 380 µL of binding mastermix from step 2 in Lysis & Bind plate.
4. Start the run on the CyBio Felix – One4All_v2.bms.
5. Follow 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).

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	Wash Buffer 1	850 µL	400 µL
Wash 2	Wash Buffer 1	850 µL	350 µL
Wash 3	80% Ethanol	350 µL	250 µL
Elution	Nuclease-free Water	60 µL	60 µL

A



B

Figure 1: Deck layout for the One4All Kit, Custom on the CyBio Felix Liquid Handler.
(A) Analytik Jena CyBio FeliX instrument with the CyBio FeliX Extraction Set, including Alpaqua® Magnum FLX™ magnet and Bioshake 3000-T elm mounted with an adapter for deep well plates. **(B)** Reagent and accessory positions for implementing purification with the One4All Kit, Custom.

Results

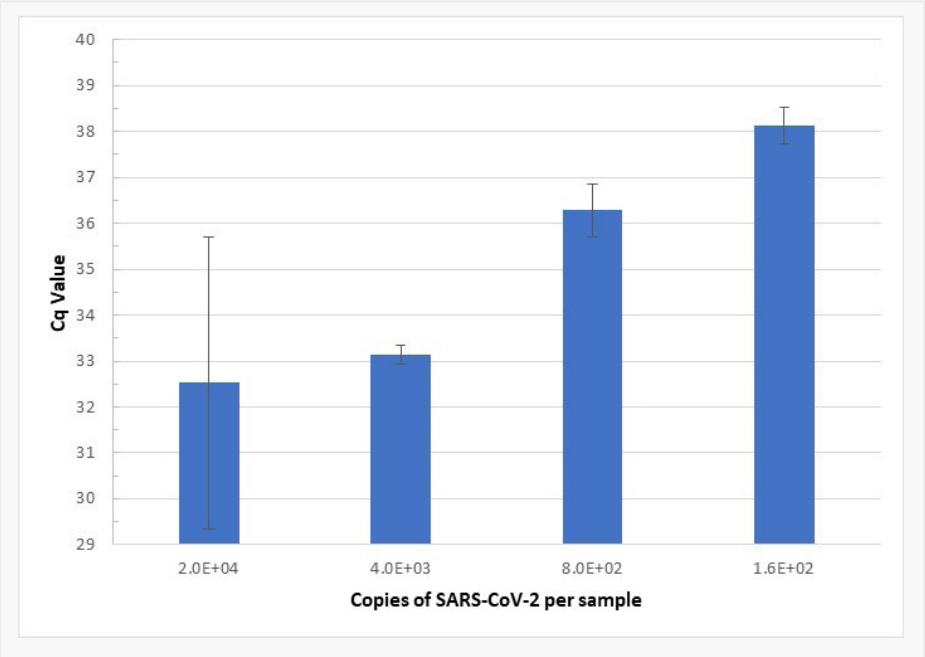


Figure 2: Detection of SARS-CoV-2 RNA Purified from VTM using the One4All Kit, Custom on the CyBio FeliX Liquid Handler. Improviral™ Viral Preservative Medium (Improve, Cat.# 8110111) was spiked with SARS-CoV-2 reconstituted from Helix Elite™ Inactivated SARS-CoV-2 Standard (Microbiologics, Cat. # HE0065N) in VTM. The stock solution (~2 x 104 copies of SARS-CoV-2 per sample) was serially diluted 1:5 in media to 4000, 800 and 160 copies per sample. No virus controls were run along with spiked samples. 200 µL of the spiked VTM was extracted with the One4All Kit, Custom (Cat.# AX9760) on the CyBio FeliX Liquid Handler, as described above. Two elution volumes were used (100 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 GoTaq® Probe 1-Step qPCR System (Cat.# A6121). Each reaction contained 5 µL of eluate with 10 µL of 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 control and no virus control samples did not amplify.

This protocol was developed by Promega Applications Scientists and is intended for research use only. Users are responsible for determining suitability of the protocol for their application.
For further information contact Technical Services at: techserv@promega.com.

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This document is true and correct at the time of publication; the information within is subject to change. Methods were developed and tested using the following software versions: CyBio Composer Version 2.70, CyBio FeliX Firmware 4.40.00, Pipetting Head Firmware CyBio-LPK 3.71.005. Other documents may supersede this document, including technical modifications and corrections.

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