Promega

Product Application

Purification of Viral RNA from Norgen Total Nucleic Acid Preservation Tubes with the Maxwell® RSC Viral Total Nucleic Acid Purification Kit

Purify viral RNA from Norgen Total Nucleic Acid Preservation Tubes using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit with the Maxwell® RSC or Maxwell® RSC 48 Instruments.

Kit: Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330)

Analyses: RT-qPCR for detection of Respiratory Syncytial Virus (RSV), Influenza A, and SARS-CoV-2.

Sample Type(s): Samples collected in Norgen Total Nucleic Acid Preservation Tubes, e.g., nasopharyngeal swabs

Input: 250µl

Materials Required:

- Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330)
- Maxwell® RSC Instrument (Cat.# AS4500) or Maxwell® RSC 48 Instrument (Cat.# AS8500)
- Heat block set to 56°C

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.

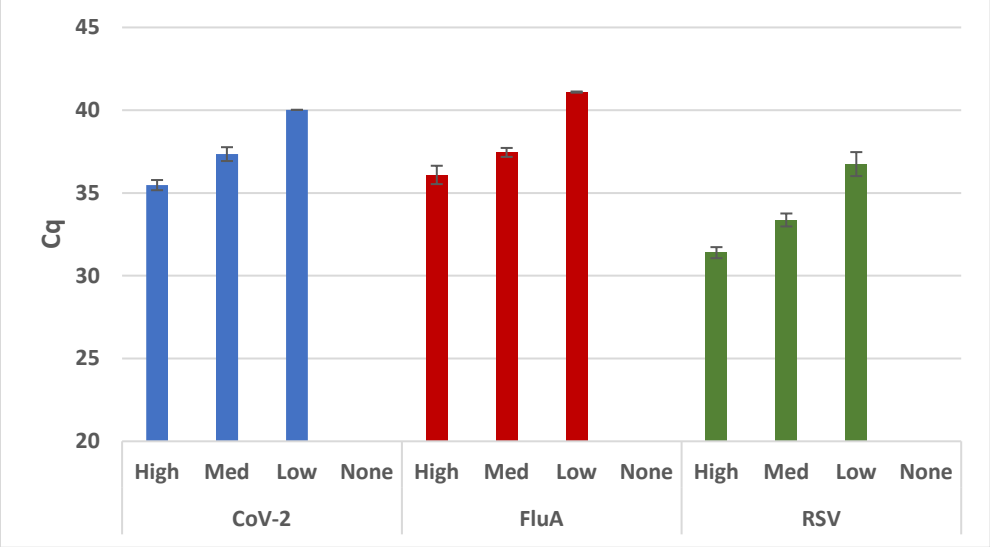
For further information, see Technical Manual TM420, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

1. Transfer 250µl of inoculated media from Norgen Total Nucleic Acid Preservation Tubes to a 1.5ml tube.
2. Add 300µl Lysis Buffer and 30µl Proteinase K to each sample. Alternatively, prepare a master mix of Lysis Buffer and Proteinase K for all samples immediately before use, and add 330µl of the master mix to each sample.
3. Vortex for 10 seconds.
4. Incubate samples at 56°C for 10 minutes.
5. Meanwhile, prepare cartridges as indicated in the Maxwell® RSC Viral Total Nucleic Acid Purification Kit Technical Manual (TM420).
    a. Add 50µl of Nuclease Free Water to elution tubes.
6. Transfer the entire lysate to well #1.
7. Select the Maxwell® RSC Viral Total Nucleic Acid run method, place the prepared deck tray in the Maxwell® RSC Instrument, and start the method.

Results:



Detection of RSV, Influenza A and SARS-CoV-2 RNA extracted from Norgen Total Nucleic Acid Preservation Tubes. Media from Total Nucleic Acid Preservation Tubes was spiked with SARS-CoV-2, RSV A and Influenza A virus reconstituted from Helix Elite™ Inactivated Standard Inactivated Influenza A/B and Respiratory Syncytial Virus (Microbiologics Cat.# HE0044) and Helix Elite™ Inactivated Standard Inactivated SARS-CoV-2 Whole Virus (Microbiologics Cat.# HE0065) in 1X PBS. High virus sample contained approximately 9.2 x 10^3 copies of SARS-CoV2, 1.7 x 10^5 copies of RSV A, and 7.5 x 10^4 copies of Influenza A per sample. Medium virus sample is a 1:10 dilution of the high virus sample in media. Low virus sample is a 1:10 dilution of the medium virus sample in media. 250µl of the spiked media was extracted with Maxwell® RSC Viral Total Nucleic Acid Purification Kit on the Maxwell® RSC 48 Instrument as described above. Following nucleic acid purification, presence of SARS-CoV-2, RSV A, and Influenza A was detected by RT-qPCR using the GoTaq® 1-Step Probe qPCR System (Cat.# A6121). Each reaction contained 5µl of eluate with 12.5µl of the GoTaq® Probe qPCR Master Mix with dUTP, 0.5µl of GoScript™ RT Mix for 1-Step RT-qPCR, and Nuclease-Free Water added to a final volume of 25µl. For RSV[1] or Influenza A[2], 1000nM forward and reverse primers and 200nM probe were used. For SARS-CoV-2[3], 400nM forward and reverse primers and 200nM probe were used. 1-step RT-qPCR thermal cycling was as follows: reverse transcription at 45°C for 15 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. Signal acquisition was during the annealing/extension stage of cycling. Data represent the average of duplicate purifications amplified in duplicate. Error bars indicate standard deviation of n=4. For Influenza A (Low), 2 of 4 replicates did not amplify. For SARS-CoV-2 (Low), 3 of 4 replicates did not amplify.

References:

1. Fry, A.M., *et al.*, (2010) The Burden of Hospitalized Lower Respiratory Tract Infection due to Respiratory Syncytial Virus in Rural Thailand, *PLoS One*. **5**, e15098.

2. Selvaraju, S.B., *et al.*, (2010). Evaluation of Three Influenza A and B Real-Time Reverse Transcription-PCR Assays and a New 2009 H1N1 Assay for Detection of Influenza Viruses, *Journal of Clinical Microbiology.* **48**, 3870-3875.

3. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel EUA. CDC-006- 00019, Revision: 05, Effective 07/13/2020.