Promega

Product Application

Automated Purification of Viral RNA from Nasal Swab Specimens Collected in VTM, PBS, and Saline

Purify viral RNA from nasal swab specimens collected in viral transport media (VTM), phosphate buffered saline (PBS) or saline using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit on the Maxwell® RSC Instrument.

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

Analyses: RT-qPCR

Sample Type(s): Viral specimens collected in VTM[1], PBS or saline

Input: 200µl

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 200µl of viral specimen in VTM, PBS, or saline to a 1.5ml tube.
2. Add 200µl of Lysis Buffer and 20µl of Proteinase K to each sample. Alternatively, prepare a master mix of Lysis Buffer and Proteinase K for all samples immediately before use, and add 220µl 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:

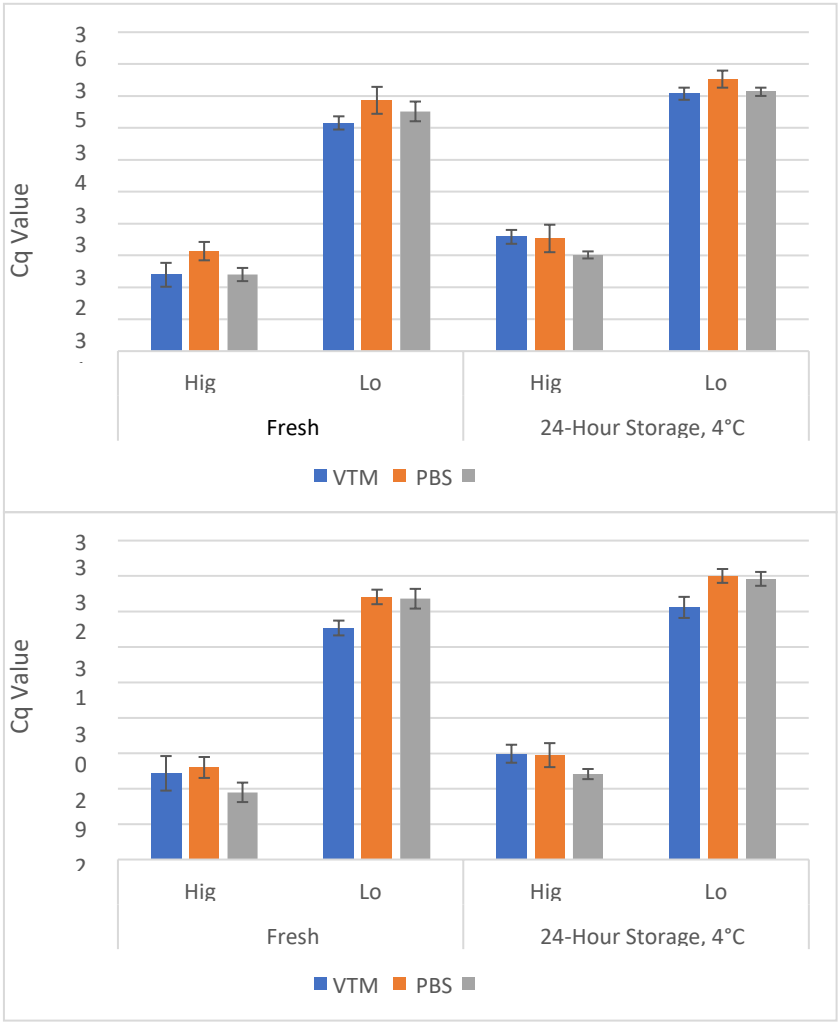

Figure 1. Detection of MS2 and RSV RNA extracted from viral specimens collected in viral transport media (VTM), PBS, or saline. VTM[1], PBS (1X, pH 7.0), or saline was inoculated with a nasal swab and spiked with MS2 bacteriophage and RSV A virus reconstituted from Helix Elite™ Inactivated Standard Inactivated Influenza A/B and Respiratory Syncytial Virus (Microbiologics Cat.# HE0044N). High virus sample contains approximately 1 x 10^7 copies of MS2 and 7.5 x 10^4 copies of RSV A per 200µl sample. Low virus sample is a 1:25 dilution of the high virus sample. 200µl of the spiked viral samples, fresh or stored at 4°C for 24 hours, was extracted with Maxwell® RSC Viral Total Nucleic Acid Purification Kit on the Maxwell® RSC Instrument as described above. Presence of MS2 (*top panel*) and RSV A (*bottom panel*) was detected by RT-qPCR using the GoTaq® Probe 1-Step RT-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, 900nM forward and reverse primers and 250nM probe (MS2) or 1000nM forward and reverse primers and 200nM probe (RSV), and Nuclease-Free Water added to a final volume of 25µl. 1-step RT-qPCR thermal cycling was as follows: reverse transcription at 50°C for 30 minutes, hot-start activation at 95°C for 2 minutes, and then 45 cycles of denaturation at 95°C for 15 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 triplicate purifications amplified in duplicate. Error bars indicate standard deviation of n=6.

Reference:

1. Centers for Disease Control and Prevention. (2020). Preparation of Viral Transport Medium. SOP# DSR-052-01. Retrieved from https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf.