Promega

Product Application

Automated Purification of Viral RNA from PrimeStore® Molecular Transport Medium (MTM)

Purify viral RNA from PrimeStore® Molecular Transport Medium 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 B, and MS2.

Sample Type(s): Samples collected in MTM, e.g., nasopharyngeal swabs

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 inoculated MTM 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 10 seconds.
4. Incubate samples at 56°C for 10 minutes.
5. Meanwhile, prepare cartridges as indicated in the Maxwell® 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:

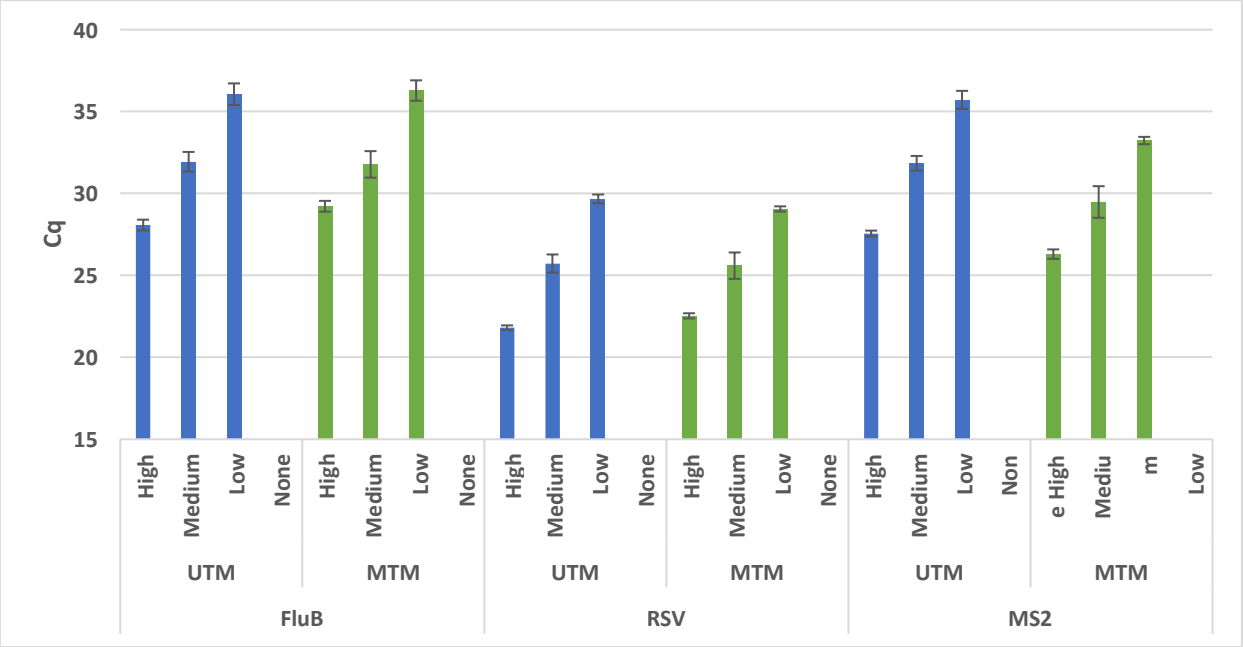

Figure 1. Detection of RSV, Influenza B and MS2 RNA extracted from MTM. MTM or Universal Transport Medium for Virus (UTM) was inoculated with a nasopharyngeal swab and spiked with purified MS2 virus as well as RSV A and Influenza B (Hong Kong) virus reconstituted from Helix Elite™ Inactivated Standard Inactivated Influenza A/B and Respiratory Syncytial Virus (Microbiologics Cat.# HE0044N) in 1X PBS. High virus sample contains approximately 1 x 10^7 copies of MS2 and 2 x 10^5 copies each of Influenza B and RSV A per sample. Medium virus sample is a 1:10 dilution of the high virus sample in MTM or UTM. Low virus sample is a 1:10 dilution of the medium virus sample in MTM or UTM. 200µl of the spiked UTM 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 MS2, RSV A, and Influenza B was detected by RT-qPCR using 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 B[2], 1000nM forward and reverse primers and 200nM probe were used. For MS2, 900nM forward and reverse primers and 250nM probe were used. For RSV[1] or Influenza B[2], 1-step RT-qPCR thermal cycling was as follows[2]: 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. For MS2, 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 15 seconds and annealing/extension at 60°C for 60 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.

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.