Promega

Product Application

Automated Purification of Viral RNA from Samples in Collected in CDC Viral Transport Medium

Purify viral RNA from samples collected in CDC Viral Transport Medium using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit with the Maxwell® RSC or Maxwell® RSC 48 Instrument.

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

Analyses: RT-qPCR for detection of Respiratory Syncytial Virus (RSV) and Influenza B

Sample Type(s): Samples collected in Viral Transport Medium, e.g., nasopharyngeal swabs

Input: 200µl

Materials Required:

- Viral Transport Medium (VTM) - see CDC SOP# DSR-052-01 for a detailed procedure for production of VTM
- 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.

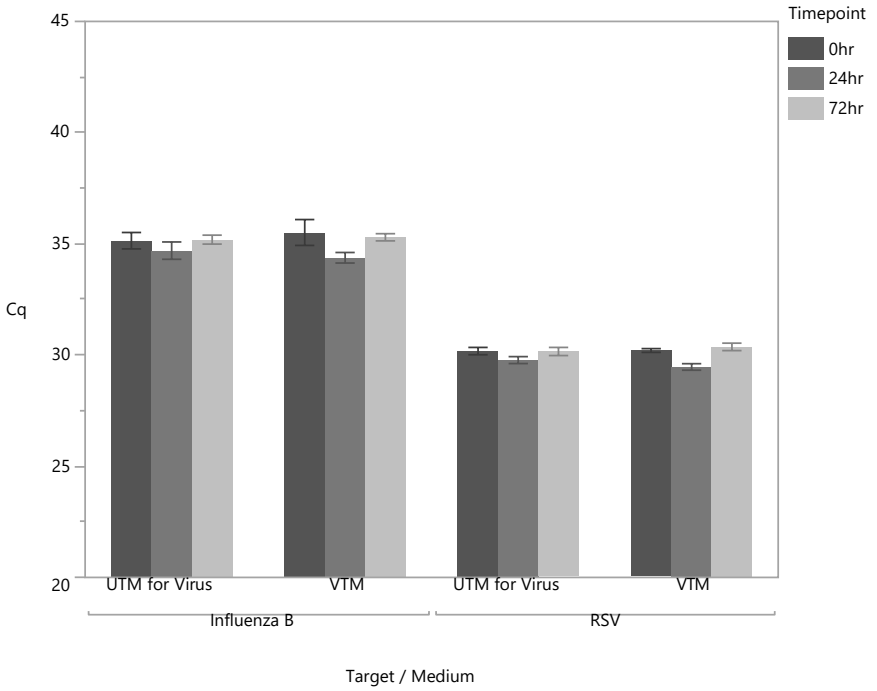
For further information, see Technical Manual TM420, available at: **www.promega.com/protocols**

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

Protocol:

1. Transfer 200µl of inoculated Viral Transport Medium 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® 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 and Influenza B RNA extracted from UTM® for Virus or Viral Transport Medium (VTM). UTM® or VTM were inoculated with a nasopharyngeal swab, spiked with 2 x 10^4 copies/200µl sample of RSV A and Influenza B virus reconstituted in 1X PBS from Helix Elite™ Inactivated Standard Inactivated Influenza A/B and Respiratory Syncytial Virus (Microbiologics Cat.# HE0044N), and stored at 4°C until use. Viral RNA from 200µl of the spiked UTM® or VTM was extracted on separate days at 0 hours, 24 hours, and 72 hours with Maxwell® RSC Viral Total Nucleic Acid Purification Kit on the Maxwell® RSC Instrument as described above. Purified RNA was frozen at -80°C and thawed once prior to amplification. Presence of RSV A and Influenza B 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, 1000nM forward and reverse primers and 200nM probe for RSV[2] or Influenza B[3], and Nuclease-Free Water added to a final volume of 25µl. 1- step RT-qPCR thermal cycling was as follows[3]: 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.

References:

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.
2. 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.
3. 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.