

Purification of Viral RNA from Universal Transport Medium for Virus with the Maxwell® RSC Viral Total Nucleic Acid Purification Kit

Purify viral RNA from Universal Transport Medium (UTM®) for Virus 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) and Influenza B.

Sample Type(s): Samples collected in UTM® for Virus, 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.

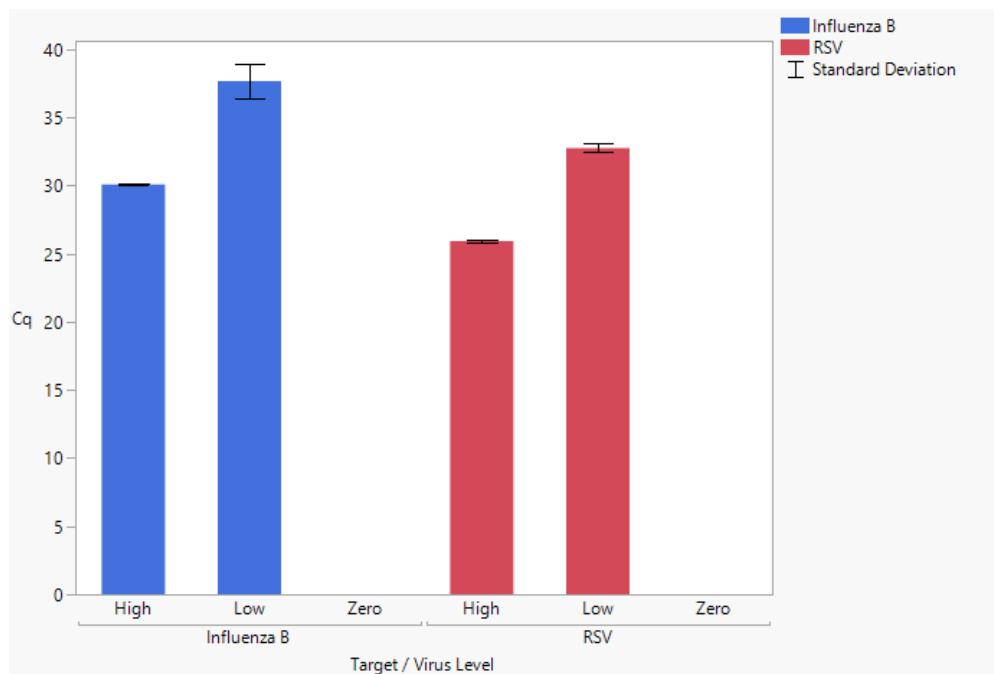
For further information, see Technical Manual TM420, available at:

www.promega.com/protocols

or contact Technical Services at: techserv@promega.com

Protocol:

1. Transfer 200µl of inoculated UTM® for Virus to a 1.5ml tube.
2. Add 200µl Lysis Buffer and 20µl 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 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 (UTM). UTM was inoculated with a nasopharyngeal swab and spiked with 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 UTM. High virus sample contains approximately 2×10^5 copies each of Influenza B and RSV A per sample. Low virus sample is a 1:100 dilution of the high virus sample in UTM. 200 μ l of the spiked UTM was extracted with Maxwell® RSC Viral Total Nucleic Acid Purification Kit on the Maxwell® RSC Instrument as described above. Following nucleic acid purification, presence of RSV A and Influenza B was detected by RT-qPCR using GoTaq® 1-Step Probe qPCR System (Cat.# A6121). Each reaction contained 5 μ l of eluate with 12.5 μ l of the GoTaq® Probe qPCR Master Mix with dUTP, 0.5 μ l of GoScript™ RT Mix for 1-Step RT-qPCR, 1000nM forward and reverse primers and 200nM probe for RSV¹ or Influenza B², and Nuclease-Free Water added to a final volume of 25 μ l. 1-step RT-qPCR thermal cycling was as follows²: 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 duplicate purifications amplified in quadruplicate across two RT-qPCR experiments. Error bars indicate standard deviation of n=8.

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.