

Purification of Viral RNA from Sputum with the Maxwell® RSC Viral Total Nucleic Acid Purification Kit

Purify viral RNA from sputum 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): Sputum

Input: 200µl

Materials Required:

- DTT, Molecular Grade (Cat.# V3151)
- Nuclease-Free Water (Cat.# P1193)
- PBS, pH 7.2 (Gibco Cat.# 20012027) or similar
- 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

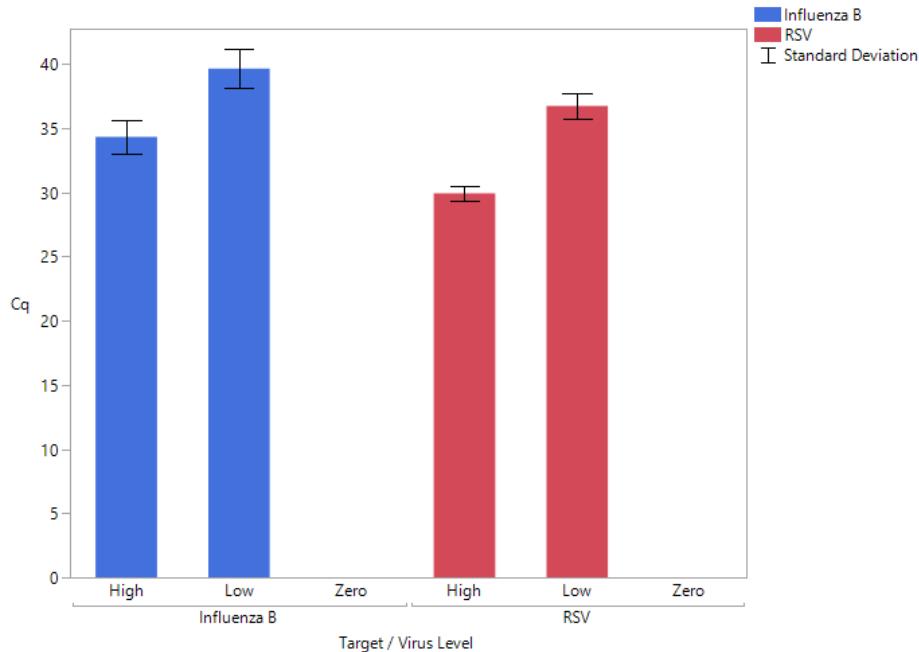
Sputum Processing¹:

1. Weigh appropriate amount of DTT, and rehydrate in Nuclease-Free Water to 500mM final concentration. Mix gently by pipetting to dissolve. DTT must be freshly made.
2. Prepare a 1:51 dilution of the 500mM DTT in PBS, pH 7.2. For example, add 100µl of 500mM DTT to 5.0ml of PBS.
3. Add an equal volume of diluted DTT in PBS to the sputum sample.
4. Incubate at room temperature with intermittent mixing by inversion until liquified or for a maximum of 30 minutes.
5. Proceed with purification.

Purification Protocol:

1. Transfer 200µl of DTT-treated sputum 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 for 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 sputum. Sputum was treated with diluted DTT in PBS for 30 minutes. RSV A and Influenza B (Hong Kong) virus were reconstituted from Helix Elite™ Inactivated Standard Inactivated Influenza A/B and Respiratory Syncytial Virus (Microbiologics Cat.# HE0044N) and spiked into treated sputum. High virus sample contains approximately 2×10^5 copies of Influenza B and RSV A per 200 μ l sample. Low virus sample is a 1:100 dilution of the high virus sample in treated sputum. 200 μ l of the spiked sputum was processed with the 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 RSV² or Influenza B³, and Nuclease-Free Water added to a final volume of 25 μ l. 1-step RT-qPCR thermal cycling was as follows³: 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. Error bars indicate the standard deviation of n=8.

References:

1. Processing of Sputum Specimens for Nucleic Acid Extraction, Centers for Disease Control
<https://www.cdc.gov/coronavirus/2019-ncov/downloads/processing-sputum-specimens.pdf>
Accessed 3/12/2020.
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.