Promega

Product Application

Purification of Viral RNA from Sputum with the Maxwell® 16 Viral Total Nucleic Acid Purification Kit

Purify viral RNA from sputum using the Maxwell® 16 Viral Total Nucleic Acid Purification Kit with the Maxwell® 16 or Maxwell® 16 MDx Instruments.

Kit: Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Cat.# AS1150)

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

Sample Type(s): Sputum

Input: 200µl

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 Bulletin TB385, available at: **www.promega.com/protocols**

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

Materials Required:

- DTT, Molecular Grade (Cat.# V3151)
- Nuclease-Free Water (Cat.# P1193)
- PBS, pH 7.2 (Gibco Cat.# 20012027) or similar
- Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Cat.# AS1150)
- Maxwell® 16 MDx Instrument (Cat.# AS3000), Maxwell® 16 Instrument (Cat.# AS2000), or Maxwell® 16 Instrument (Cat.# AS1000)
    - Configured with low elution volume (LEV) hardware
    - Firmware v4.61 or higher (Cat.# AS1000, AS2000)
    - Firmware v1.05 or higher (Cat.# AS3000)
- Heat block set to 56°C

Sputum Processing[1]:

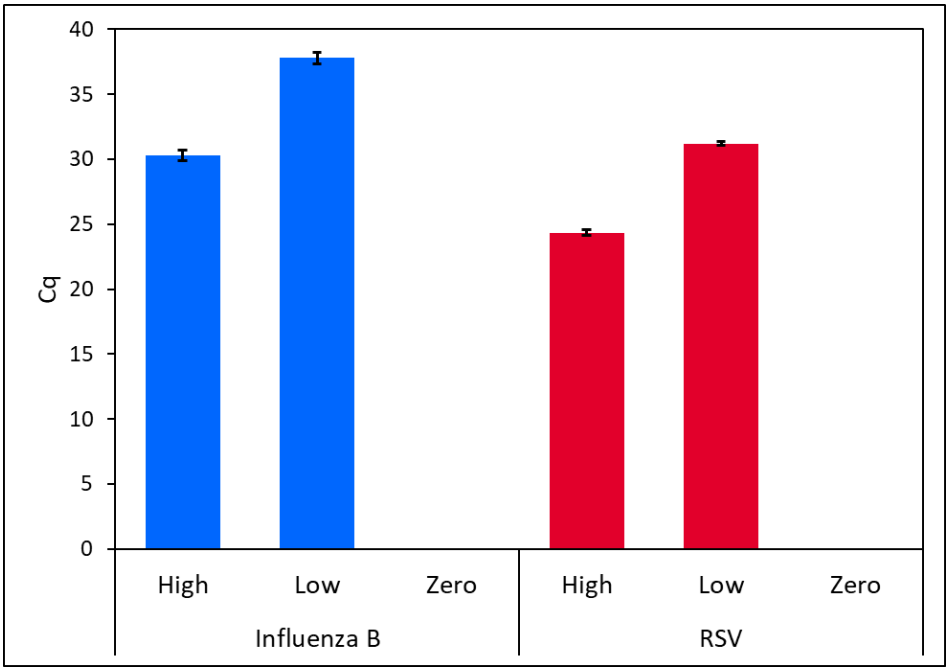
1. Weigh appropriate amount of DTT, and rehydrate in Nuclease-Free Water to 500mM final concentration. Mix gently by pipetting to dissolve. DTT must be freshly made.
2. Prepare a 1:51 dilution of the 500mM DTT in PBS, pH 7.2. For example, add 100µl of 500mM DTT to 5.0ml of PBS.
3. Add an equal volume 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. Prepare fresh Lysis Solution by combining 200µl of Lysis Buffer and 20µl Proteinase K Solution per sample plus 20% extra to compensate for pipetting losses.

2. Transfer 200µl of DTT-treated sputum to a 1.5ml tube.
3. Add 220µl of prepared Lysis Solution to each sample.
4. Close tubes and vortex for 10 seconds.
5. Incubate samples at 56°C for 10 minutes.
6. Meanwhile, prepare cartridges as indicated in the technical bulletin (TB385).
    a. Add 50µl of Nuclease-Free Water to Elution Tubes.
7. Transfer the entire lysate to well #1 of the cartridge.
8. Set up the Maxwell® 16 instrument as described in the technical bulletin (TB385). Refer to the technical manual of the instrument being used for further instrument-specific details.
9. Select the Viral protocol and follow the instrument prompts. After opening the door, place the prepared Maxwell® 16 LEV Cartridge Rack in the instrument, and start the protocol.

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 x 10^5 copies of Influenza B and RSV A per 200µl sample. Low virus sample is a 1:100 dilution of the high virus sample in treated sputum. 200µl of the spiked sputum was processed with the Maxwell® 16 Viral Total Nucleic Acid Purification Kit on the Maxwell® 16 MDx 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 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 the standard deviation.

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.