Promega

Product Application

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

Purify viral RNA from stabilized saliva 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): Stabilized saliva stored in Oragene•RNA (RE-100) or Oragene•DNA (OG-500) tubes (DNA Genotek)

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**

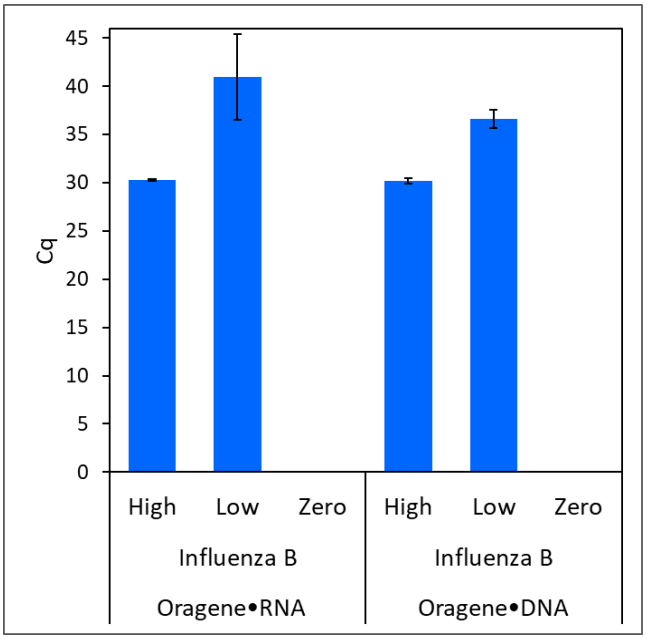
or contact Technical Services at: **techserv@promega.com**

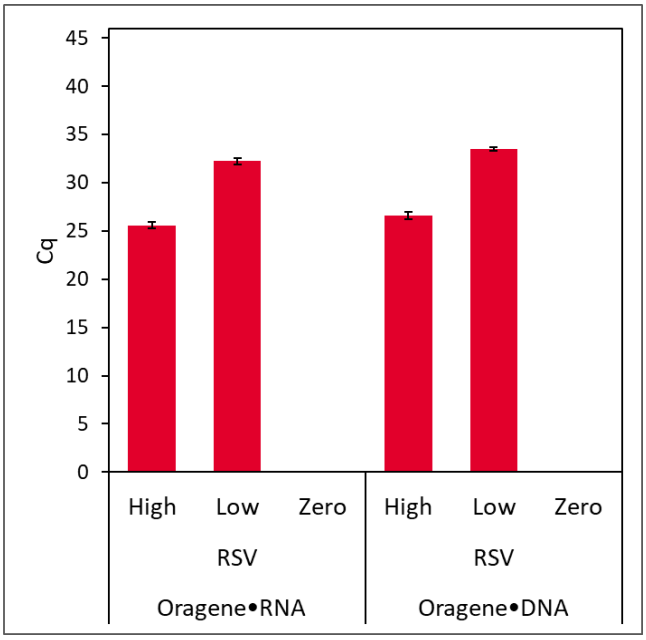
Protocol:

1. Transfer 200µl of stabilized saliva 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.

Note: It is recommended that no more than 100ng of total nucleic acid be input into GoTaq® 1-Step Probe RT-qPCR System (Cat.# A6121) reactions. Addition of too much total nucleic acid to RT-qPCR reactions can inhibit detection of the intended viral target.

Results:





Detection of RSV and Influenza B RNA extracted from stabilized saliva. Saliva was collected in Oragene•RNA (RE-100) or Oragene•DNA (OG-500) tubes (DNA Genotek) from four individuals and incubated overnight at room temperature. Stabilized saliva from each tube type was pooled. 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 stabilized saliva from each tube type. 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 stabilized saliva. 200µl of the spiked stabilized saliva was processed 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 RT-qPCR System (Cat.# A6121). Each reaction contained 5µl of undiluted 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[1] or Influenza B[2], and Nuclease-Free Water added to a final volume of 25µl. 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, with signal acquisition during the annealing/extension stage of cycling. Data represent the average of duplicate purifications amplified in duplicate. Error bars indicate the standard deviation. Influenza B was detected in 2 of 4 replicates for low virus samples from the Oragene•RNA tubes and the Oragene•DNA tubes.

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.