Promega

Product Application

Automated Purification of Viral RNA from Saliva Collected in ORAcollect®•RNA Collection Devices

Purify viral RNA from saliva collected in Oragene•RNA collection devices 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

Sample Type(s): Saliva collected in ORAcollect®•RNA collection devices

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 Manual TM420, available at: **www.promega.com/protocols**

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

Materials Required:

- Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330)
- ORAcollect®•RNA (DNA GenoTek, Cat.# OR-100)
- Neutralizer solution (DNA GenoTek, Cat.# RE-LZN)
- Maxwell® RSC Instrument (Cat.# AS4500) or Maxwell® RSC 48 Instrument (Cat.# AS8500)
- Heat block set to 90°C and 56°C

Protocol:

1. Collect samples in ORAcollect®•RNA collection device according to manufacturer's instructions. Invert 15 times to mix saliva with the buffer.
2. Transfer 200µl of saliva/buffer sample to a 1.5ml tube.
3. Incubate samples at 90°C for 15 minutes, then cool to room temperature.
4. Add 1/25 volume (8µl) of Neutralizer solution. Incubate on ice for 10 minutes.
5. Centrifuge at maximum speed (>13,000 x g) for 3 minutes.
6. Taking care not to disturb the pellet, carefully remove the supernatant to a fresh 1.5ml tube.
7. 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.
8. Vortex 10 seconds.
9. Incubate samples at 56°C for 10 minutes.
10. Meanwhile, prepare cartridges as indicated in the technical manual (TM420). Add 50µl of Nuclease-Free Water to elution tubes.
11. Transfer the entire lysate to well #1.

12. 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:

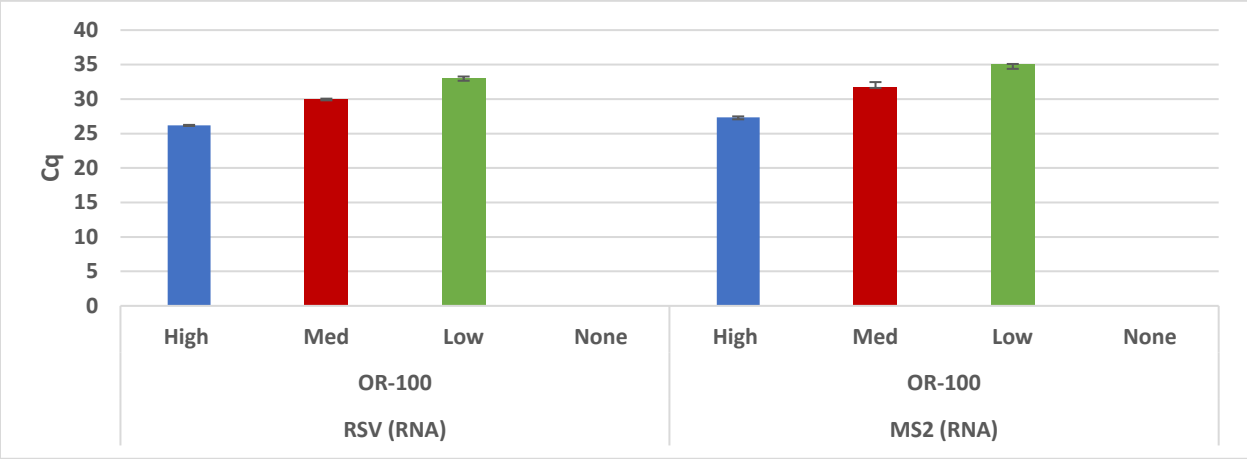

Figure 1. Detection of RSV and MS2 RNA extracted from saliva in ORAcollect®•RNA collection devices. Saliva was spiked with RSV A reconstituted from Helix Elite™ Inactivated Standard Inactivated Influenza A/B and Respiratory Syncytial Virus (Microbiologics, Cat.# HE0044N) and MS2 virus (Zeptometrix, Cat.# 0810066). High virus sample contains approximately 2 x 10^5 copies of RSV A and 1 x 10^7 copies of MS2 per sample. Medium virus sample is a 1:10 dilution of the high virus sample in saliva/buffer. Low virus sample is a 1:10 dilution of the medium virus sample in saliva/buffer. 200µl of the spiked saliva was extracted with the Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330) on the Maxwell® RSC Instrument (Cat.# AS4500) as described above. Following nucleic acid purification, presence of RSV A and MS2 was detected by RT-qPCR using the GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6121). For RSV[1], 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, 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. For MS2, each reaction contained 2µl of eluate with 10µl of the GoTaq® Probe qPCR Master Mix with dUTP, 0.4µl of GoScript™ RT Mix for 1-Step RT-qPCR, 900nM forward and reverse primers and 250nM probe, and Nuclease-Free Water added to a final volume of 20µl. 1-step RT-qPCR thermal cycling was as follows: reverse transcription at 45°C for 15 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 60°C for 1 minute. Signal acquisition occurred during the annealing/extension stage of cycling. Data represent the average of duplicate purifications amplified in duplicate. Error bars indicate standard deviation of n=4.

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.