Promega

Product Application

Automated Purification of Viral TNA from Saliva Collected in OMNIgene®•ORAL Collection Devices

Purify viral RNA from saliva collected in OMNIgene®•ORAL 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 OMNIgene®•ORAL collection devices (Cat.# OM-505 from DNA Genotek*)

Input: 200µl

Materials Required:

- Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330)
- OMNIgene®•ORAL (Cat.# OM-505 from DNA Genotek*)
- Maxwell® RSC Instrument (Cat.# AS4500) or Maxwell® RSC 48 Instrument (Cat.# AS8500)
- Heat block or water bath set to 50°C and 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. Collect samples in OMNIgene®•ORAL collection device according to manufacturer's instructions. Shake the tube for 10 seconds to mix saliva with the buffer.
2. Incubate samples at 50°C for 1 hour in a water bath or for 2 hours in an air incubator.
3. Transfer 200µl of saliva/buffer sample to a 1.5ml tube.
4. 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.
5. Vortex 10 seconds.
6. Incubate samples at 56°C for 10 minutes.
7. Meanwhile, prepare cartridges as indicated in the technical manual (TM420). Add 50µl of Nuclease-Free Water to elution tubes.
8. Transfer the entire lysate to well #1.
9. Select the Maxwell® RSC Viral Total Nucleic Acid run method, place the prepared deck tray in the Maxwell® RSC Instrument, and start the method.

* OMNIgene®•ORAL is For Research Use Only, not for use in diagnostic procedures. ®OMNIgene is a registered trademark of DNA Genotek Inc.

Results:

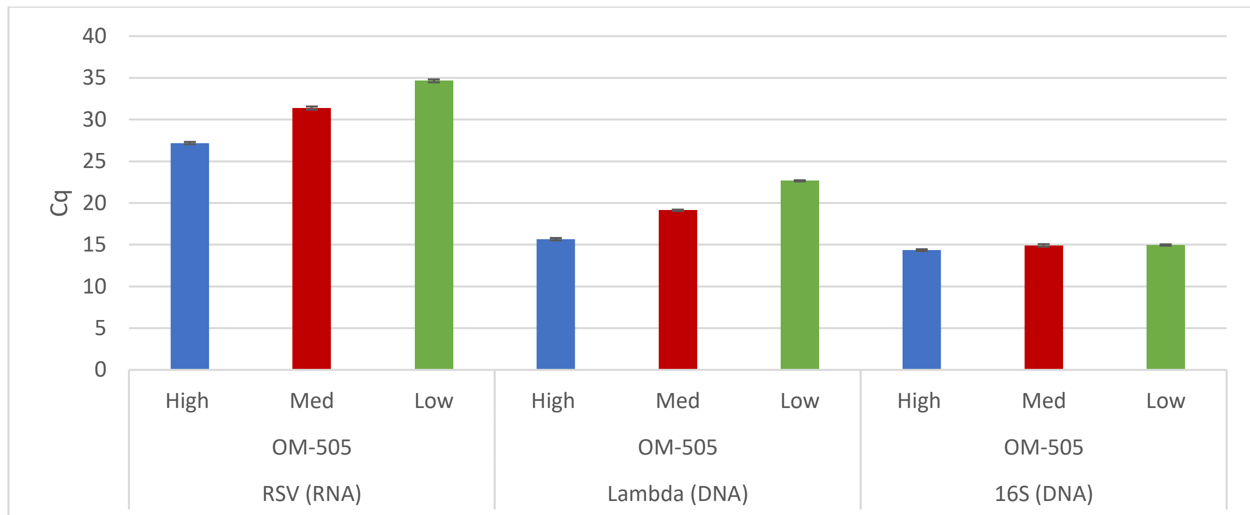

Figure 1. Detection of RSV RNA, Lambda DNA, and 16S rRNA DNA extracted from saliva in OMNIgene®•ORAL collection devices. Saliva was spiked with RSV A reconstituted from Helix Elite™ Inactivated Standard Inactivated Influenza A/B and Respiratory Syncytial Virus (Microbiologics, Cat.# HE0044N) and Lambda virus. High virus sample contains approximately 2 x 10^5 copies of RSV A and 1 x 10^7 copies of Lambda 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[1] was detected by RT-qPCR using the GoTaq® Probe 1-Step RT-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, 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. Presence of Lambda was detected by qPCR using GoTaq® Probe qPCR System (Cat.# A6101). Each reaction contained 2µl of eluate with 10µl of GoTaq® Probe qPCR Master Mix, 900nM forward and reverse primers and 250nM probe, and Nuclease-Free Water added to a final volume of 20µl. qPCR thermal cycling was as follows: 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. Presence of 16S rRNA DNA was detected by qPCR using GoTaq® qPCR System (Cat.# A6001). Each reaction contained 2µl of eluate with 10µl of GoTaq® qPCR Master Mix, 1µM forward and reverse primers, and Nuclease-Free Water added to a final volume of 20µl. qPCR thermal cycling was as follows: hot-start activation at 95°C for 2 minutes, and then 45 cycles of denaturation at 95°C for 10 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. 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.