

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:

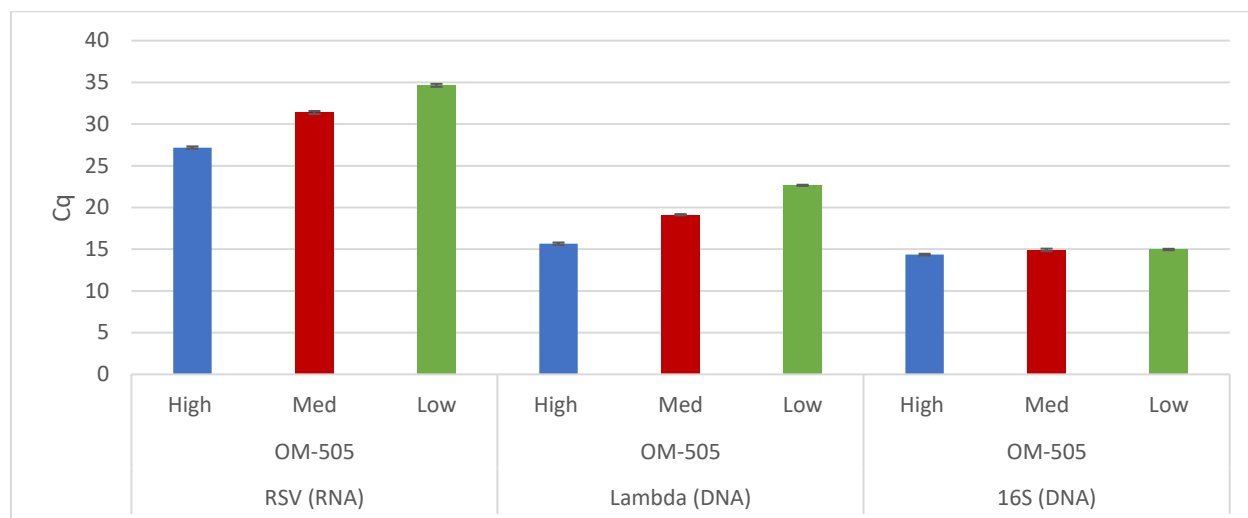


Figure 1. Detection of RSV RNA, Lambda DNA, and 16S rRNA DNA extracted from saliva in OMNigene®•ORAL collection devices. Saliva was spiked with RSV A reconstituted from Helix Elite™ Inactivated Standard Inactivated Influenza A/B and Respiratory Syncytial Virus (Microbiologics, Cat.# HE0044N) and Lambda virus. High virus sample contains approximately 2×10^5 copies of RSV A and 1×10^7 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 A¹ 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 follows²: 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.