Promega

# Product Application

## Purification of Viral RNA from Stabilized Saliva with the ReliaPrep™ Viral Total Nucleic Acid Purification Kit, Custom

*Purify viral RNA from stabilized saliva using the ReliaPrep™ Viral Total Nucleic Acid Purification Kit, Custom.*

**Kit:** ReliaPrep™ Viral Total Nucleic Acid Purification Kit, Custom (Cat.# AX4820)

**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:**

- ReliaPrep™ Viral Total Nucleic Acid Purification Kit, Custom (Cat.# AX4820)
- Microcentrifuge
- 1.5ml tubes
- Heat block set to 56°C
- Isopropanol

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 TM330, available at: **www.promega.com/protocols**

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

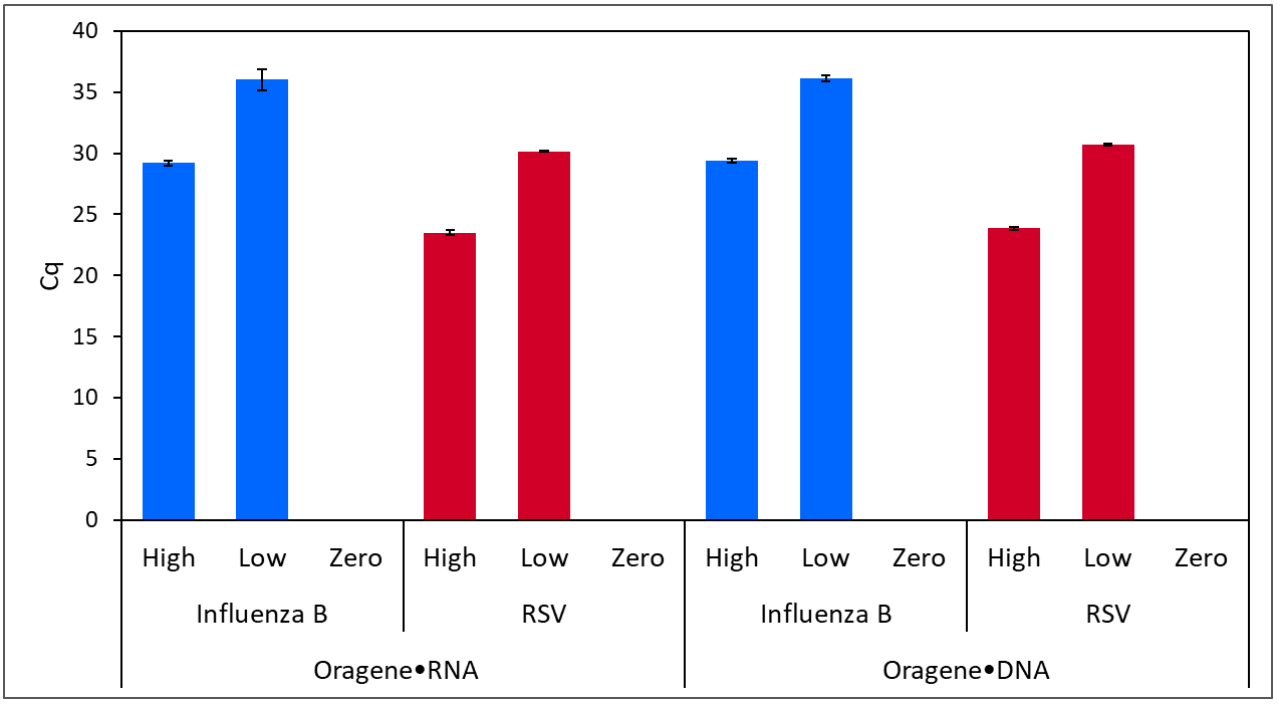
**Protocol**:

Binding Buffer (BBA) provided with this kit is not required for processing stabilized saliva stored in Oragene•RNA (RE-100) or Oragene•DNA (OG-500) tubes.

1. Add 20µl of Proteinase K (PK) Solution to each 1.5ml tube.
2. Transfer 200µl of stabilized saliva to each 1.5ml tube.
3. Add 200µl of Cell Lysis Buffer (CLD) to each tube. Vortex for 10 seconds.
4. Incubate samples at 56°C for 10 minutes.
5. While samples are incubating, place a ReliaPrep™ Binding Column into an empty Collection Tube.
6. Remove the tube from the heat block. Add 250µl of Isopropanol. Vortex for 10 seconds.
7. Transfer the tube contents to the ReliaPrep™ Binding Column, cap it and place it in a microcentrifuge.
8. Centrifuge for 1 minute at maximum speed.
9. Remove the collection tube containing flowthrough, and discard the liquid.
10. Place the binding column into a new collection tube.

11. Add 500µl of Column Wash Solution (CWD) to the column, and centrifuge for 3 minutes at maximum speed. Discard the flowthrough.
12. Repeat Step 11 twice for a total of three washes.
13. Place the column in a clean 1.5ml tube.
14. Add 60µl of Nuclease-Free Water to the column. Centrifuge for 1 minute at maximum speed.
15. Save the eluate, and discard the ReliaPrep™ Binding Column.

**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 the ReliaPrep™ Viral Total Nucleic Acid Purification Kit, Custom 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 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.

**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.