Promega

Product Application

Purification of Viral RNA from Universal Transport Medium for Virus with the ReliaPrep™ Blood gDNA Miniprep System

Purify viral RNA from Universal Transport Medium (UTM®) for Virus using the ReliaPrep™ Blood gDNA Miniprep System.

Kit: ReliaPrep™ Blood gDNA Miniprep System (Cat.# A5081, A5082)

Analyses: RT-qPCR for detection of Respiratory Syncytial Virus (RSV) and Influenza B

Sample Type(s): Samples collected in UTM® for Virus, e.g. nasopharyngeal swabs

Input: 200µl

Materials Required:

- ReliaPrep™ Blood gDNA Miniprep System (Cat.# A5081 or A5082)
- 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**

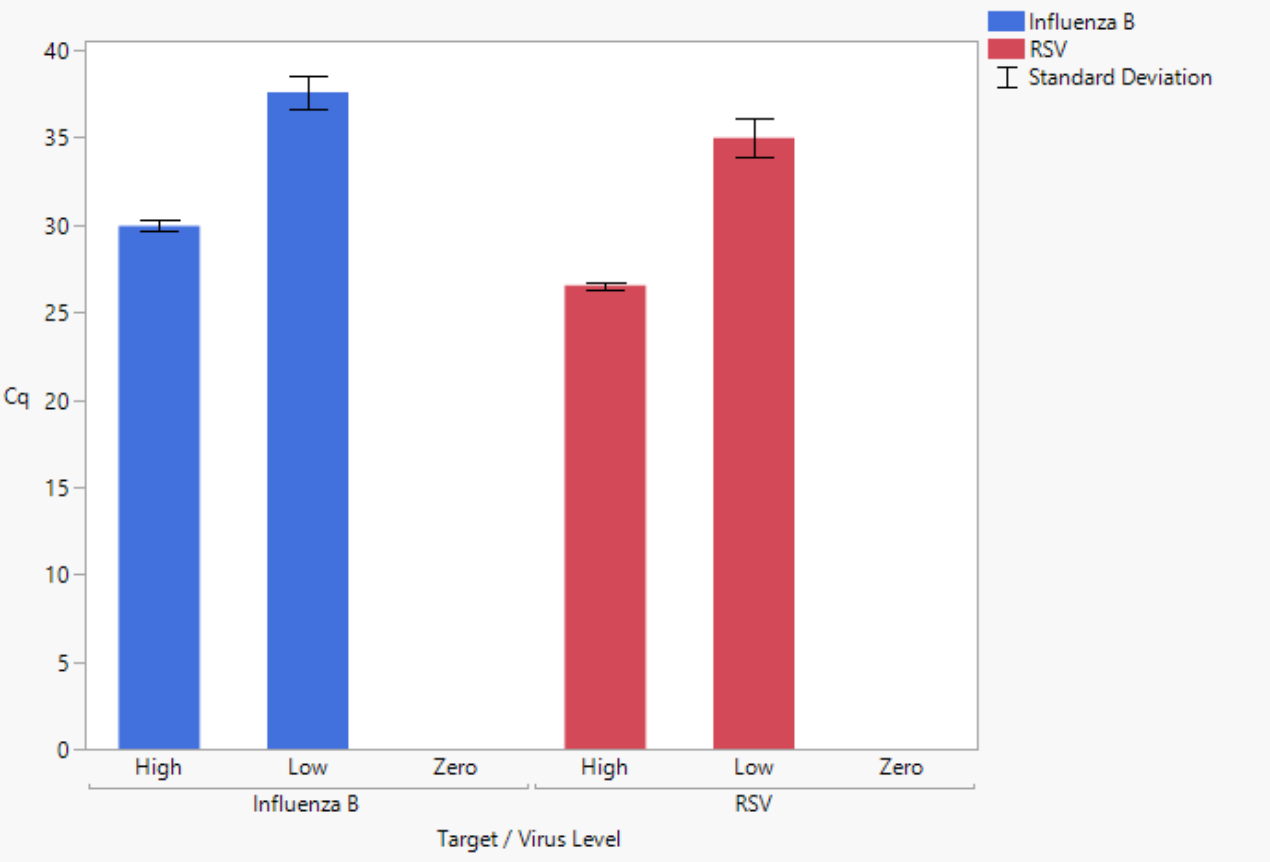
Protocol:

Binding Buffer (BBA) provided with this kit is not required for processing samples in UTM® for Virus.

1. Add 20µl of Proteinase K (PK) Solution to each 1.5ml tube.
2. Transfer 200µl of inoculated UTM® for Virus 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 UTM® for Virus (UTM). UTM was inoculated with a nasopharyngeal swab and spiked with RSV A and Influenza B (Hong Kong) virus reconstituted from Helix Elite™ Inactivated Standard Inactivated Influenza A/B and Respiratory Syncytial Virus (Microbiologics Cat.# HE0044N) in UTM. High virus sample contains approximately 2 x 10^5 copies each of Influenza B and RSV A per sample. Low virus sample is a 1:100 dilution of the high virus sample in UTM. 200µl of the spiked UTM was extracted with ReliaPrep™ Blood gDNA Miniprep System as described above. Following nucleic acid purification, presence of RSV A and Influenza B was detected by RT-qPCR using GoTaq® 1-Step Probe 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 extractions 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.