Promega

Product Application

Purification of Viral RNA from Sputum with the ReliaPrep™ Blood gDNA Miniprep System

Purify viral RNA from sputum 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): Sputum

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

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

Materials Required:

- DTT, Molecular Grade (Cat.# V3151)
- Nuclease-Free Water (Cat.# P1193)
- PBS, pH 7.2 (Gibco Cat.# 20012027) or similar
- ReliaPrep™ Blood gDNA Miniprep System (Cat.# A5081 or A5082)
- Microcentrifuge
- 1.5ml tubes
- Heat block set to 56°C
- 100% Isopropanol

Sputum Processing:

1. Weigh appropriate amount of DTT, and rehydrate in Nuclease-Free Water to 50mM final concentration. Mix gently by pipetting to dissolve. DTT must be freshly made.
2. Prepare a 1:50 dilution of the 50mM DTT in PBS, pH 7.2 (1mM DTT final concentration). For example, add 100µl of 50mM DTT to 4.9ml of PBS.
3. Add an equal volume of 1mM DTT in PBS to the sputum sample.
4. Incubate at room temperature with intermittent mixing by inversion until liquified or for a maximum of 30 minutes.
5. Proceed with purification.

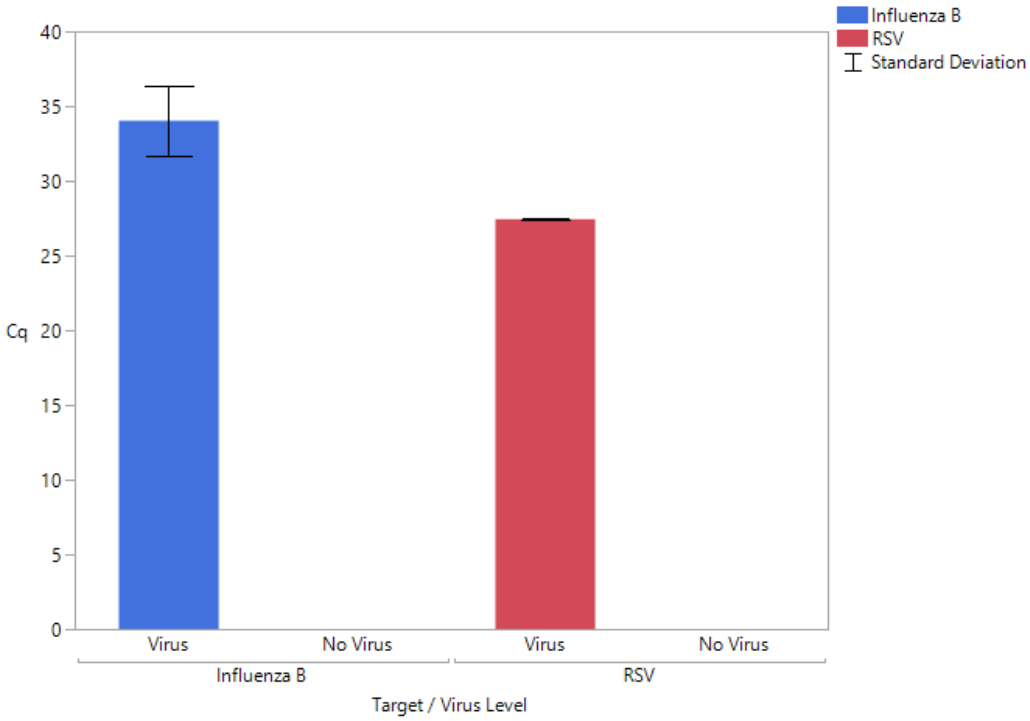
Purification Protocol:

Binding Buffer (BBA) provided with this kit is not required for processing sputum samples.

1. Add 20µl of Proteinase K (PK) Solution to each 1.5ml tube.
2. Transfer 200µl of DTT-treated sputum 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 100% 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 sputum. Sputum was treated with 1mM DTT in PBS for 30 minutes. 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 treated sputum at approximately 2 x 10^5 copies of each virus per 200µl sample. 200µl of the spiked sputum was processed with the 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 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.