Promega

Product Application

Purification of Viral RNA from Universal Transport Medium for Virus with the Maxwell® HT Viral TNA Kit, Custom

Purify viral RNA from Universal Transport Medium (UTM®) for Virus using the Maxwell® HT Viral TNA Kit, Custom manually in 96-well plate format.

Kit: Maxwell® HT Viral TNA Kit, Custom (Cat.# AX2340)

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

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

Materials Required:

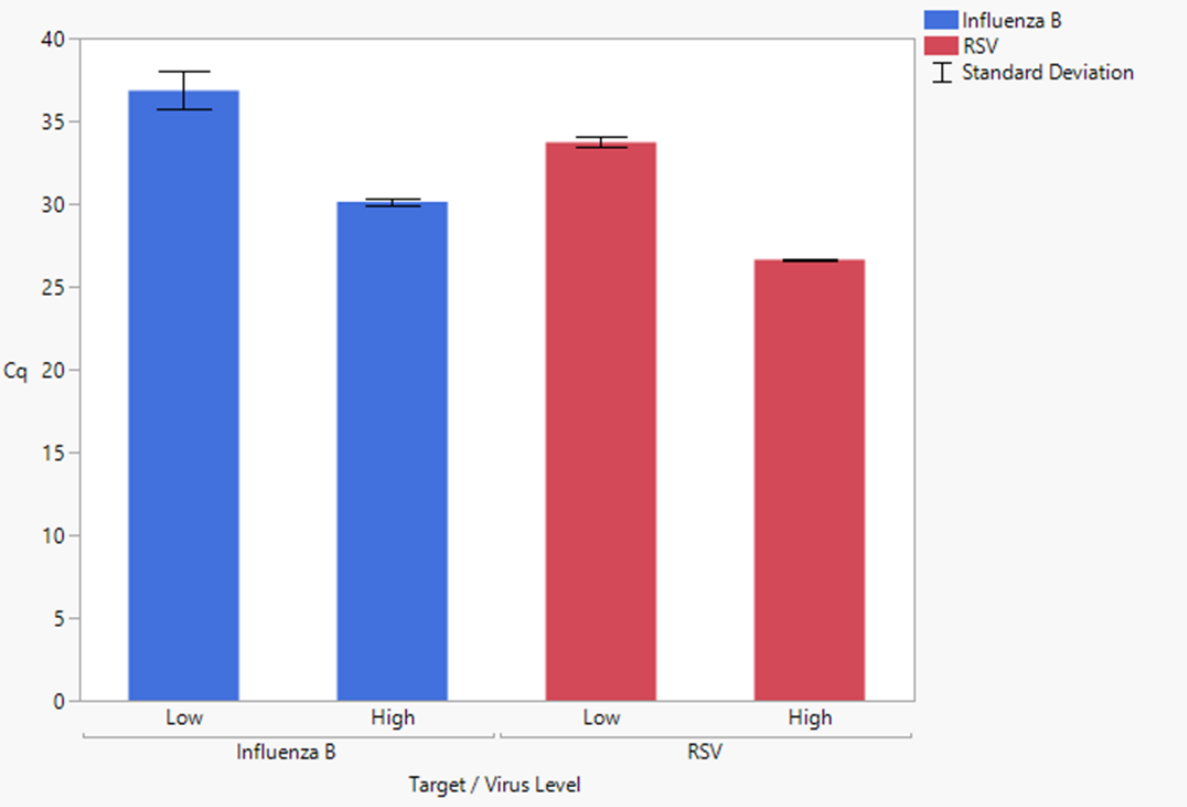
- Maxwell® HT Viral TNA Kit, Custom (Cat.# AX2340)
- 4/40 Wash Solution (Cat.# A2221)
- Alcohol Wash, Blood (Cat.# MD1411)
- 80% Ethanol
- 100% Isopropanol
- MagnaBot® FLEX 96 Magnetic Separation Device (Cat.# VA1290), or similar
- Nunc™ 96-Well Polypropylene DeepWell™ Storage Plates (ThermoFisher Scientific Cat.# 95040452), or similar
- Microseal 'B' PCR Plate Sealing Film (Bio-Rad, Cat.# MSB 1001), or similar
- 96-well plate heater with adapter appropriate for heat transfer for 96-well deep well plate
- Plate shaker (3mm diameter orbit)

Protocol:

1. Prepare 4/40 Wash Buffer and Alcohol Wash Buffer as indicated on the bottles.
2. Add the following reagents to each well of the 96-well plate*:
   a. 200µl of Lysis Buffer
   b. 35µl of Proteinase K
   *Optional: A master mix of the above reagents (plus at least 10% overage) can be prepared immediately before sample addition and 235µl added to each well.
3. Transfer 200µl of inoculated UTM® for Virus to each well of the 96-well plate. Apply a plate seal.
4. Incubate for 15 minutes at 65°C on a 96-well plate heater fitted with an adapter compatible with a 96-well deep well plate. The adapter should have heating elements that extend between the wells for efficient heat transfer.

5. Remove the plate seal, and add^ 530µl of 100% Isopropanol and 35µl of MagneSil® RED resin (vortex vigorously to resuspend prior to addition) to each well of the 96-well plate.
   ^Optional: A master mix of the above reagents (plus at least 10% overage) can be prepared and 565µl added to each well. Vortex master mix vigorously before adding to wells.
6. Apply a new plate seal, and immediately shake at 900rpm for 15 minutes at room temperature on a plate shaker (3mm orbit).
7. Tip mix 10 times with a P1000 pipette. Capture resin on a MagnaBot® FLEX 96 Magnetic Separation Device (MagnaBot® magnet) for 1 minute. Discard lysate.
8. Add 900µl of 4/40 wash buffer. Resuspend resin by pipetting with a P1000 pipette.
9. Capture resin on a MagnaBot® magnet for 1 minute. Discard wash.
10. Add 450µl of alcohol wash. Resuspend resin by pipetting with a P1000 pipette.
11. Capture resin on a MagnaBot® magnet for 1 minute. Discard wash.
12. Add 450µl of 80% EtOH. Resuspend resin by pipetting with a P1000 pipette.
13. Capture resin on a MagnaBot® magnet for 1 minute. Discard wash.
14. Remove excess ethanol with a P20 pipette. Dry for 5 minutes at room temperature.
15. Elute for 5 minutes at room temperature by adding 60µl of Nuclease-Free Water, tip mixing 10 times after addition and then again 10 times after 2.5 minutes.
16. Transfer eluates to a clean 96-well plate or to tubes.

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 Maxwell® HT Viral TNA Kit, Custom manually in a 96-well plate 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 with 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.