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 a microtube 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:

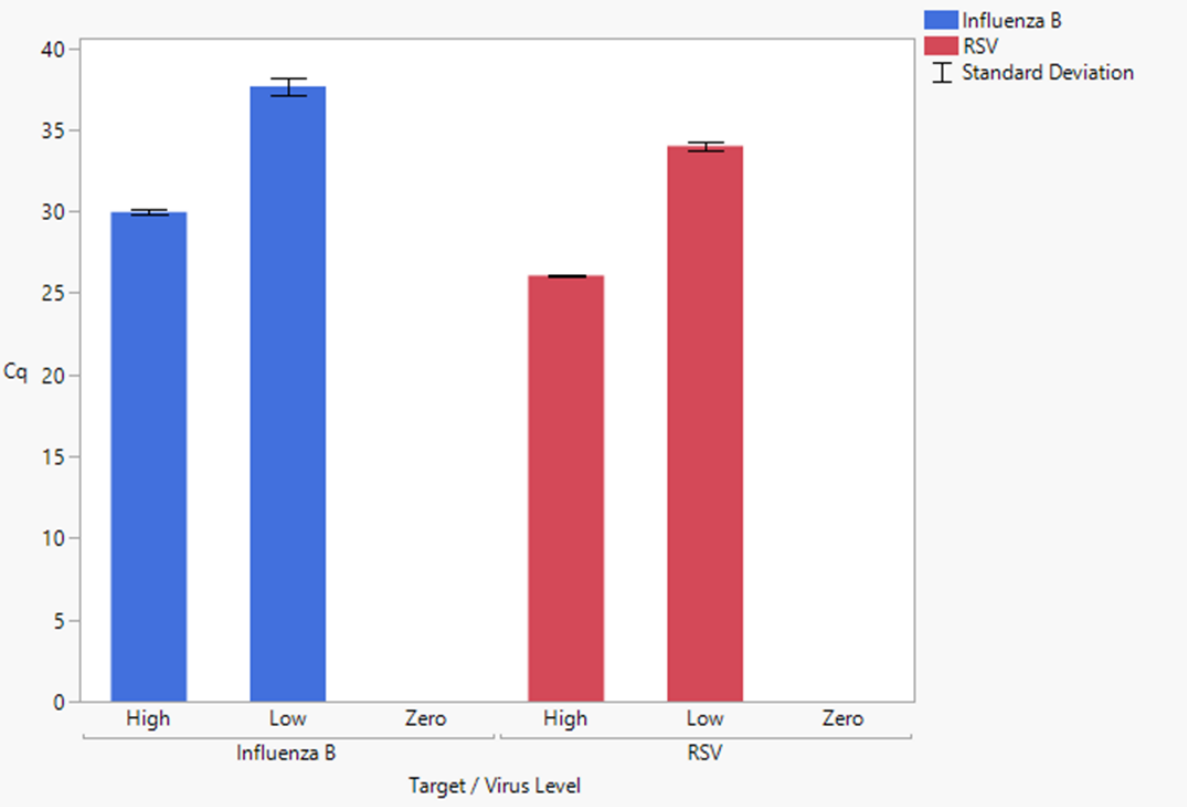
- Maxwell® HT Viral TNA Kit, Custom (Cat.# AX2340)
- 4/40 Wash Solution (Cat.# A2221)
- Alcohol Wash, Blood (Cat.# MD1411)
- 80% Ethanol
- 100% Isopropanol
- MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5342)
- 1.5ml tubes
- Heat block set to 56°C
- Thermomixer at room temperature

Protocol:

1. Prepare 4/40 Wash Buffer and Alcohol Wash Buffer as indicated on the bottles.
2. Transfer 200µl of inoculated UTM® for Virus to a 1.5ml tube.
3. Add the following reagents to each tube*:
   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.
4. Vortex for 10 seconds to mix.
5. Incubate at 56°C for 10 minutes.
6. Add 530µl of 100% Isopropanol and 35µl of MagneSil® RED resin (vortex vigorously to resuspend prior to addition) to each tube. Pipette up and down to mix.
   ^Optional: A master mix of the above reagents (plus at least 10% overage) can be prepared and 565µl added to each tube. Vortex master mix vigorously before adding to tubes.
7. Shake at room temperature for 15 minutes in a thermomixer set to 1,500 rpm.

8. Capture resin on a MagneSphere® Technology Magnetic Separation Stand (magnetic stand) for 1 minute. Discard lysate.
9. Add 900µl of 4/40 Wash Buffer. Pipette up and down to resuspend resin.
10. Capture resin on a magnetic stand for 1 minute. Discard wash.
11. Add 450µl of Alcohol Wash. Pipette up and down to resuspend resin.
12. Capture resin on a magnetic stand for 1 minute. Discard wash.
13. Add 450µl of 80% EtOH. Pipette up and down to resuspend resin.
14. Capture resin on a magnetic stand for 1 minute. Discard wash.
15. Remove any additional ethanol with a P200 pipette.
16. Dry for 5 minutes at room temperature.
17. Add 60µl of Nuclease-Free Water. Pipette up and down to resuspend resin.
18. Capture resin on a magnetic stand for 1 minute. Transfer eluate to a new tube.

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 1.5ml tubes 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.