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Product Application

Purification of Viral RNA from Sputum with the Maxwell® HT Viral TNA Kit, Custom

Purify viral RNA from sputum 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): 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, 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
- 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)

Sputum Processing[1]:

1. Weigh appropriate amount of DTT, and rehydrate in Nuclease-Free Water to 500mM final concentration. Mix gently by pipetting to dissolve. DTT must be freshly made.
2. Prepare a 1:51 dilution of the 500mM DTT in PBS, pH 7.2. For example, add 100µl of 500mM DTT to 5.0ml of PBS.
3. Add an equal volume of diluted 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.

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Purification 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 DTT-treated sputum 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.

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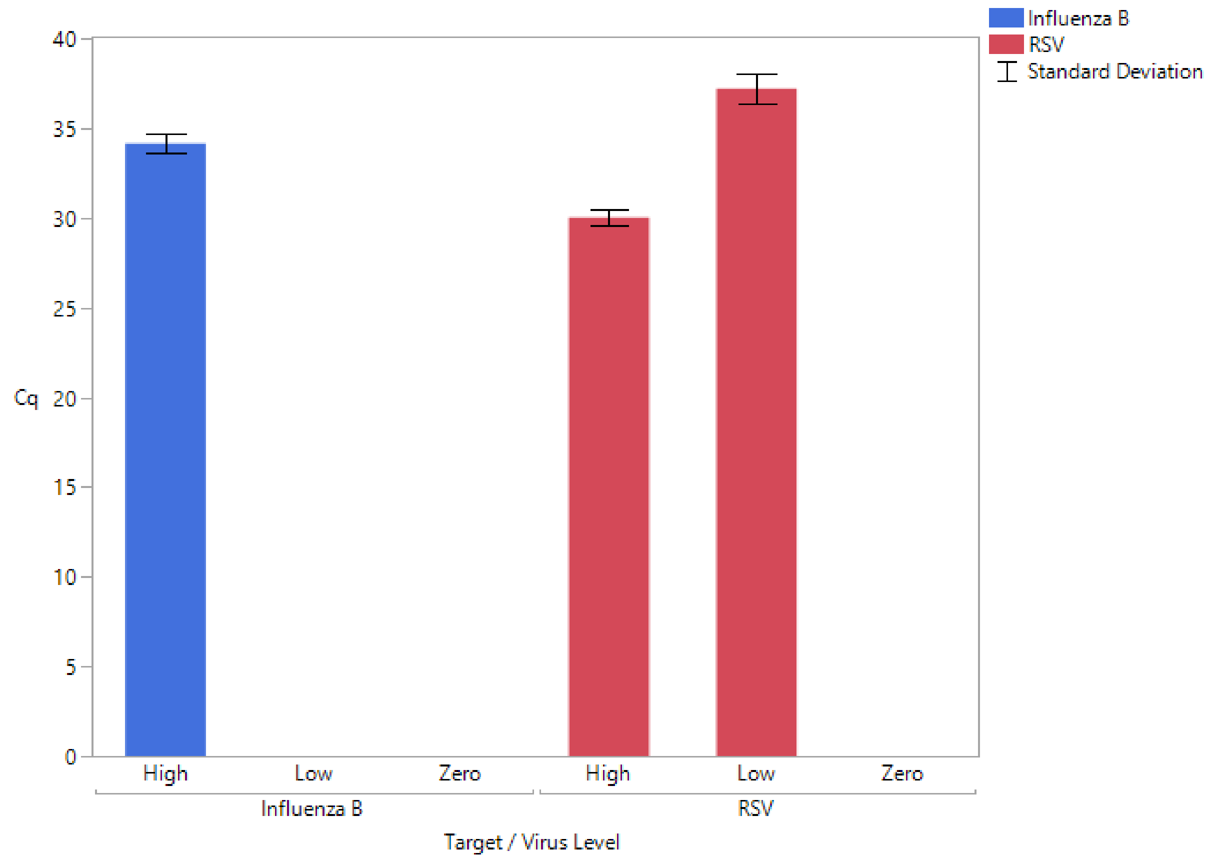
Results:



Detection of RSV and Influenza B RNA extracted from sputum. Sputum was treated with diluted 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. 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 treated sputum. 200µl of the spiked sputum was processed 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[2] or Influenza B[3], and Nuclease-Free Water added to a final volume of 25µl. 1-step RT-qPCR thermal cycling was as follows[3]: 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. Influenza B was not detected in the RNA extracted from the low virus sample.

References:

1. Processing of Sputum Specimens for Nucleic Acid Extraction, Centers for Disease Control https://www.cdc.gov/coronavirus/2019-ncov/downloads/processing-sputum-specimens.pdf Accessed 3/12/2020.

2. 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.

3. 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