

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 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):	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:
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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
- MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5342)
- 1.5ml tubes
- Heat block set to 56°C
- Thermomixer at room temperature

Sputum Processing¹:

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.

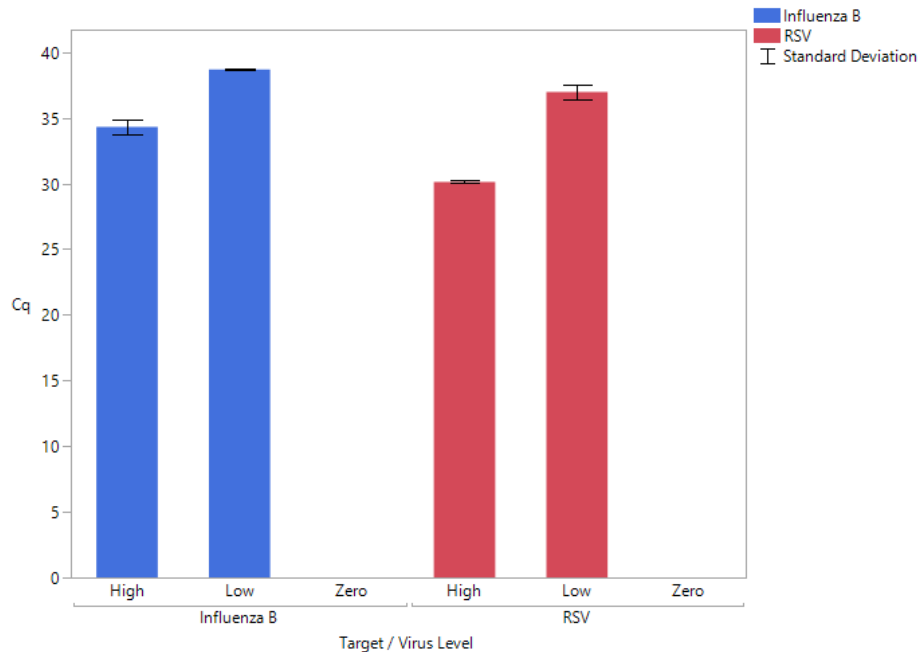
Purification Protocol:

1. Prepare 4/40 Wash Buffer and Alcohol Wash Buffer as indicated on the bottles.
2. Transfer 200µl of DTT-treated sputum 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 1,400 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 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 2×10^5 copies of Influenza B and RSV A per 200 μ l sample. Low virus sample is a 1:100 dilution of the high virus sample in treated sputum. 200 μ l of the spiked sputum was processed with the 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 5 μ l of eluate with 12.5 μ l of the GoTaq® Probe qPCR Master Mix with dUTP, 0.5 μ l of GoScript™ RT Mix for 1-Step RT-qPCR, 1000nM forward and reverse primers and 200nM probe for RSV² or Influenza B³, and Nuclease-Free Water added to a final volume of 25 μ l. 1-step RT-qPCR thermal cycling was as follows³: 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. 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.