Promega

Product Application

Equivalency Testing of the Maxwell® RSC 48 and CSC 48 Instruments with the Maxwell® RSC Viral Total Nucleic Acid Purification Kit

Using viral models to demonstrate - equivalent performance between the Maxwell® RSC 48 and Maxwell® CSC 48 Instruments.

Kit: Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330)

Analyses: RT-qPCR for detection of Respiratory Syncytial Virus (RSV), Influenza A, Influenza B, and SARS-CoV-2.

Sample Type(s): Samples collected in Viral Transport Media (VTM), e.g., nasopharyngeal swabs

Input: 200µl

Materials Required:

- Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330)
- Maxwell® RSC 48 Instrument (Cat.# AS8500) or Maxwell® CSC 48 Instrument (Cat.# AS8000)
- Heat block set to 56°C

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.

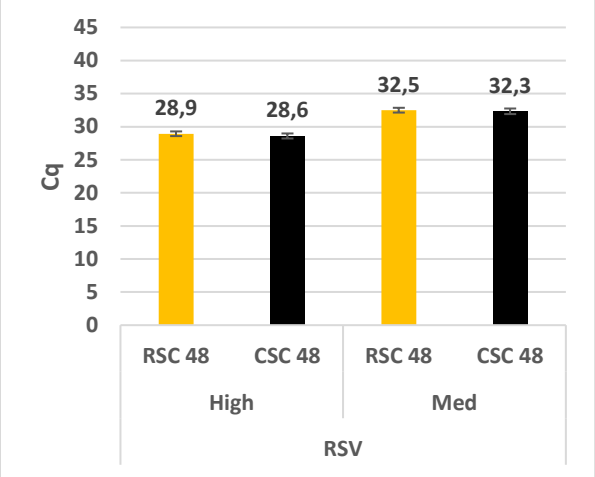
For further information, see Technical Manual TM420, available at: **www.promega.com/protocols**

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

Protocol:

1. Transfer 200µl of inoculated VTM to a 1.5ml tube.
2. Add 200µl of Lysis Buffer and 20µl of Proteinase K to each sample.
3. Vortex 10 seconds.
4. Incubate samples at 56°C for 10 minutes.
5. Meanwhile, prepare cartridges as indicated in the Maxwell® RSC Viral Total Nucleic Acid Purification Kit Technical Manual (TM420).
6. Add 50µl of Nuclease-Free Water to elution tubes.
7. Transfer the entire lysate to well #1.
8. Select the Maxwell® RSC Viral Total Nucleic Acid run method, place the prepared deck tray in the Maxwell® Instrument, and start the method.

Results:


Cq
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40
35
30
25
20
15
10
5
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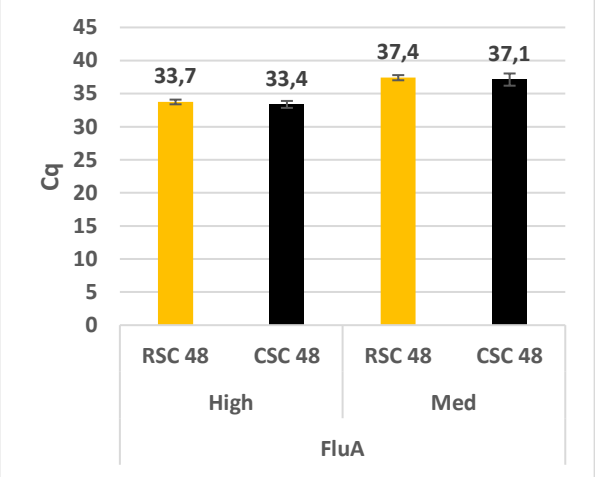
28,9
28,6
32,5
32,3
RSC 48
CSC 48
RSC 48
CSC 48
High
Med
RSV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33,7
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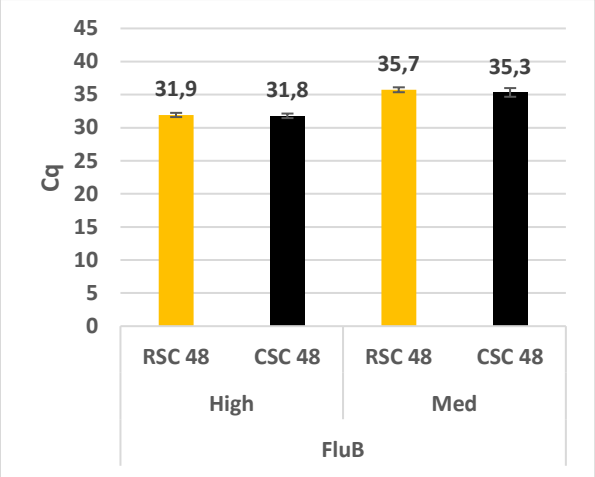
RSC 48
CSC 48
RSC 48
CSC 48
High
Med
FluA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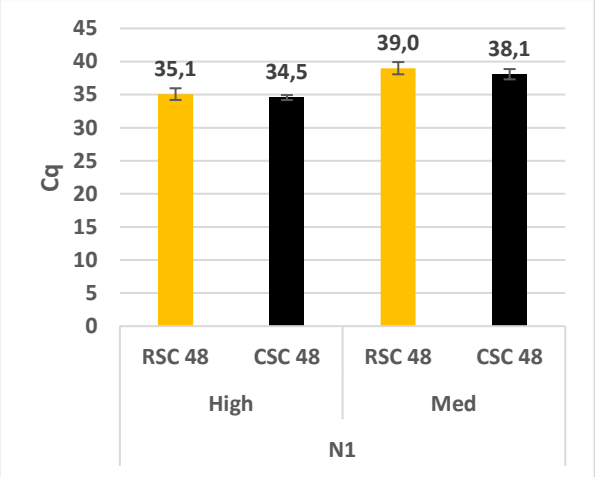
RSC 48
CSC 48
RSC 48
CSC 48
High
Med
FluB


Cq
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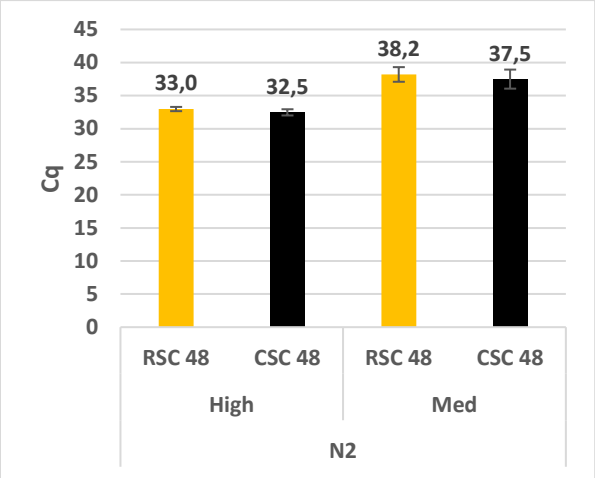
RSC 48
CSC 48
RSC 48
CSC 48
High
Med
N1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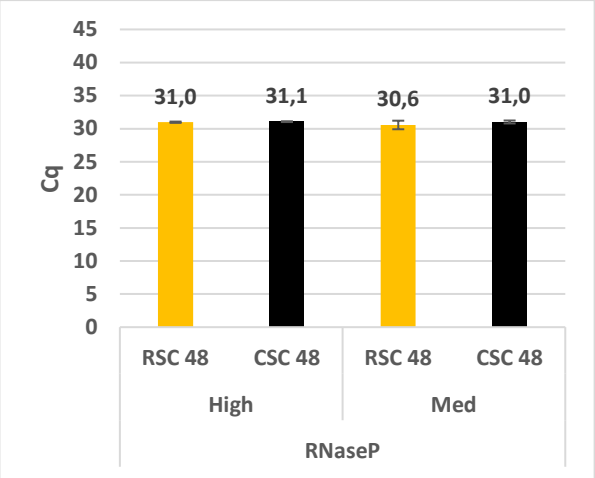
RSC 48
CSC 48
RSC 48
CSC 48
High
Med
N2


Cq
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31,0
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31,0
RSC 48
CSC 48
RSC 48
CSC 48
High
Med
RNaseP

Detection of RSV, Influenza A, Influenza B, and SARS-CoV-2 RNA extracted from VTM. VTM was spiked with SARS-CoV-2, RSV A, Influenza A, and Influenza B virus reconstituted from Helix Elite™ Inactivated Standard Inactivated Influenza A/B and Respiratory Syncytial Virus (Microbiologics Cat.# HE0044) and Helix Elite™ Inactivated Standard Inactivated SARS-CoV-2 Whole Virus (Microbiologics Cat.# HE0065) in VTM. High virus sample contained approximately 6.7 x 10^3 copies of SARS-CoV2, 1.3 x 10^4 copies of RSV A, 6 x 10^4 copies of Influenza A, and 2 x 10^4 copies of Influenza B per sample. Medium virus sample was a 1:10 dilution of the high virus sample in media. 200µl of the spiked media was extracted with the Maxwell® RSC Viral Total Nucleic Acid Purification Kit on the Maxwell® RSC 48 and CSC 48 instruments as described above. Following nucleic acid purification, presence of SARS-CoV-2, RSV A, Influenza 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, and Nuclease-Free Water added to a final volume of 25µl. For RSV[1], Influenza A[2], or Influenza B[2], 1000nM forward and reverse primers and 200nM probe were used. For SARS-CoV-2[3], 250nM forward and reverse primers and 125nM probe were used for N1 and RNaseP, and 400nM forward and reverse primers and 100nM probe were used for N2. 1-step RT-qPCR thermal cycling was as follows: reverse transcription at 45°C for 15 minutes, hot-start activation at 95°C for 2 minutes, and then 45 cycles of denaturation at 95°C for 3 seconds and annealing/extension at 55°C for 30 seconds. Signal acquisition was during the annealing/extension stage of cycling. Data represent the average of six purifications. Error bars indicate standard deviation of n=6. For the Maxwell® RSC 48, 4 of 6 replicates amplified for the N1 target at the Med level. For the Maxwell® CSC 48, 5 of 6 replicates amplified for the N1 target at the Med level.

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.

3. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel EUA. CDC-006- 00019, Revision: 05, Effective 07/13/2020.