Promega

# Product Application

## Automated Purification of RNA from Inactivated Virus in Trizol®

*Purify viral RNA from samples collected in DMEM and inactivated with Trizol® using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit with the Maxwell® RSC or Maxwell® RSC 48 Instruments.*

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

**Analyses:** RT-qPCR for detection of Respiratory Syncytial Virus (RSV) and Influenza B

**Sample Type(s):** Samples (e.g., nasopharyngeal swabs) collected in DMEM or similar and inactivated with Trizol® Reagent[1]

**Input:** 200µl of inactivated virus in Trizol® Reagent or 200µl of inactivated virus in Trizol® aqueous phase

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**

**Materials Required:**

- Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330)
- Trizol® Reagent (Ambion, Cat.# 15596026) stored at 4°C or pre-chilled on ice
- (Optional) For purification from aqueous phase
  - Chloroform, molecular biology grade
  - Centrifuge set at 4°C
- Maxwell® RSC Instrument (Cat.# AS4500) or Maxwell® RSC 48 Instrument (Cat.# AS8500)

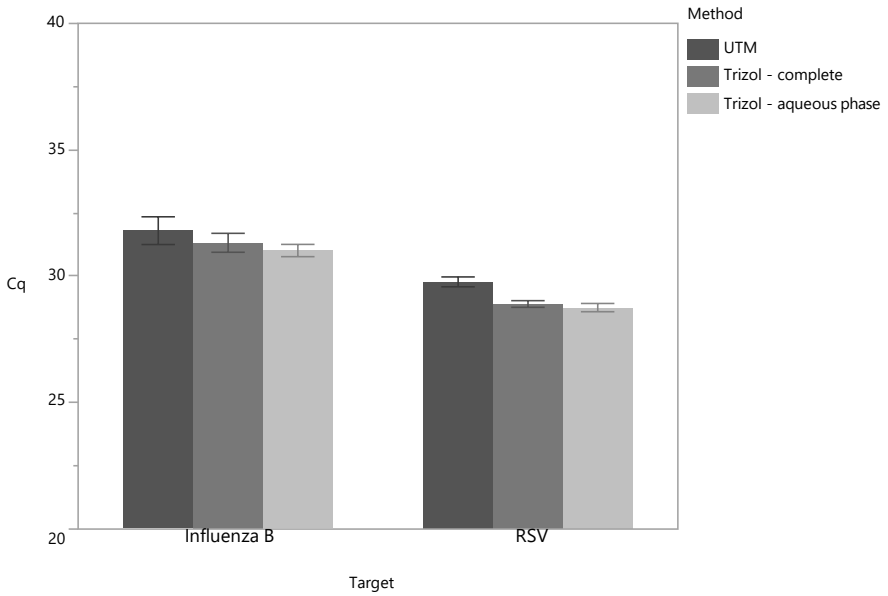
**Protocol**:

1. Add 3X volume of cold Trizol® Reagent to ≥ 200µl of inoculated sample media (e.g., DMEM).[1] Invert 5 times to mix.
2. Incubate samples at room temperature for 10 minutes to inactivate.[1] If using the sample without phase separation, invert briefly to mix and proceed to step 4.
3. (Optional) Prepare the aqueous phase as follows:
   a. Add 0.2ml Chloroform per 0.75ml Trizol® Reagent. Invert 5 times to mix.
   b. Incubate samples at room temperature for 2-3 minutes.
   c. Centrifuge at 4°C for 15 minutes at 12,000 x *g* to separate phases.
4. Transfer 200µl of inactivated virus in Trizol® Reagent or 200µl of the prepared Trizol® aqueous phase to a 1.5ml tube.
5. Add 200µl of Lysis Buffer. Vortex 10 seconds. (Note: Addition of Proteinase K Solution and heated incubation is not required for samples treated with Trizol® Reagent.)
6. Prepare cartridges as indicated in the Maxwell® RSC Viral Total Nucleic Acid Purification Kit Technical Manual (TM420).

a. 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® RSC Instrument, and start the method.

**Results:**




**Detection of RSV and Influenza B RNA extracted from viral samples inactivated with Trizol® Reagent.** Duplicate 500µl volumes of DMEM were inoculated with a nasopharyngeal swab and spiked with 3 x $10^5$ copies/sample of RSV A and Influenza B virus reconstituted in 1X PBS from Helix Elite™ Inactivated Standard Inactivated Influenza A/B and Respiratory Syncytial Virus (Microbiologics Cat.# HE0044N). Samples were pooled and 250-300µl was aliquoted into 1.5ml tubes. One aliquot was inactivated without phase separation, and the remaining two aliquots were inactivated and the aqueous phase separated as described above. Viral RNA from 200µl of the inactivated sample in complete Trizol® Reagent (3 x $10^4$ viral copies/200µl) or the aqueous phase (assumed ~6 x $10^4$ copies/200µl) was purified in triplicate with the Maxwell® RSC Viral Total Nucleic Acid Purification Kit on the Maxwell® RSC 48 Instrument as described above. For comparison, viral RNA was purified in parallel from Universal Transport Medium (UTM®) for Virus similarly inoculated with a nasopharyngeal swab and spiked with 3 x $10^5$ copies of RSV A and Influenza B per 3ml tube (2 x $10^4$ copies/200µl sample, n=3). Purified RNA was frozen at -80°C and thawed once prior to amplification. 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 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 triplicate purifications amplified in duplicate. Error bars indicate standard deviation of n=6.

**References:**

1. Kumar, M., *et al.*, (2015). Inactivation and safety testing of Middle East Respiratory Syndrome Coronavirus. *Journal of Virological Methods. 223*, 13-18.
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.