Promega

Product Application

Automated Purification of Viral RNA from Liver and Scallop

Purify viral RNA from pig liver and scallop using the Maxwell® RSC simplyRNA Tissue Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC simplyRNA Tissue Kit (Cat.# AS1340)

Analyses: RT-qPCR

Sample Type(s): Pig liver, scallop

Input: 10mg

Materials Required:

- Maxwell® RSC simplyRNA Tissue Kit (Cat.# AS1340)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Tube for homogenization
- Heat block
- Tissue homogenizer
- Vortex

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 TM416, available at: **www.promega.com/protocols**

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

Protocol:

1. Prepare Homogenization Solution + 1-Thioglycerol as described in the Maxwell® RSC simplyRNA Tissue Kit Technical Manual (TM416).
2. Homogenize 10mg sample in 200µl chilled Homogenization Solution + 1-Thioglycerol.
3. Heat homogenate at 70°C for 2 minutes.
4. Allow sample to cool for 1 minute before processing.
5. Add 200µl Lysis Buffer to 200µl tissue homogenate.
6. Vortex for 15 seconds to mix.
7. Transfer all 400µl of lysate to well #1 of the Maxwell® RSC simplyRNA Tissue cartridge.
8. Add 10µl blue DNase I solution to well #4 of the cartridge.
9. Place a plunger in well #8 of the cartridge.
10. Place an elution tube with 50µl Nuclease-Free Water on the Maxwell® RSC deck tray.
11. Load the deck tray onto the Maxwell® RSC Instrument.
12. Select and run the Maxwell® RSC simplyRNA Tissue Method.
13. Store purified RNA at -70°C.

Results: Linear detection of MS2 bacteriophage was observed across a 4-log range in both sample types. No qPCR inhibition was observed in scallop eluates, while slight inhibition was observed in liver eluates, potentially due to high total RNA concentration.

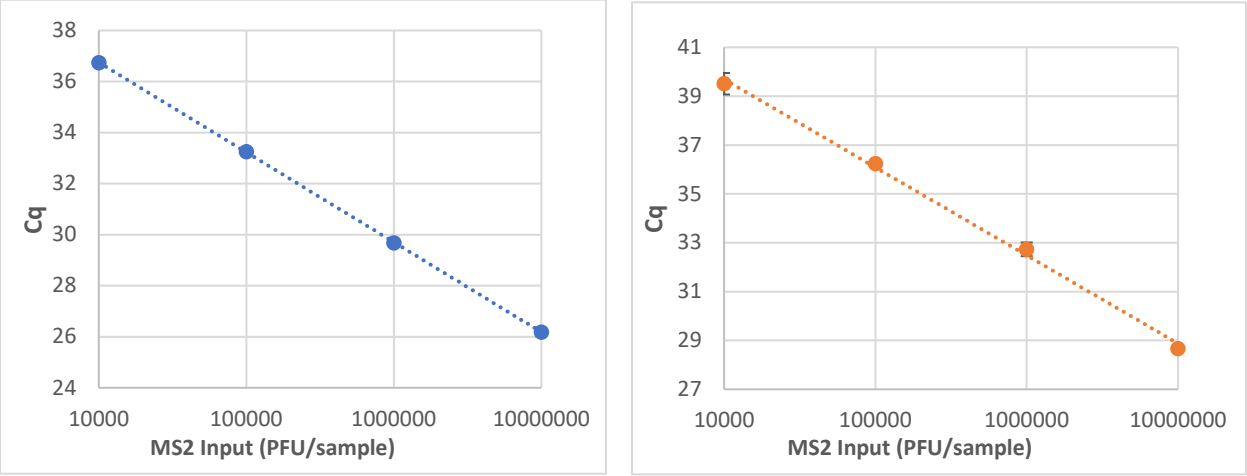

Figure 1. Average Cq values from 10mg of pig liver (left) or scallop (right) samples spiked with MS2 bacteriophage across a 4-log range. Samples were purified using the Maxwell® RSC simplyRNA Tissue Kit on the Maxwell® RSC Instrument and amplified using the GoTaq® 1-Step RT-qPCR System (Cat.# A6120) with MS2-specific primers/probes. Samples showed linear detection across a 4-log range. Data are represented as mean ± standard deviation for n=3.

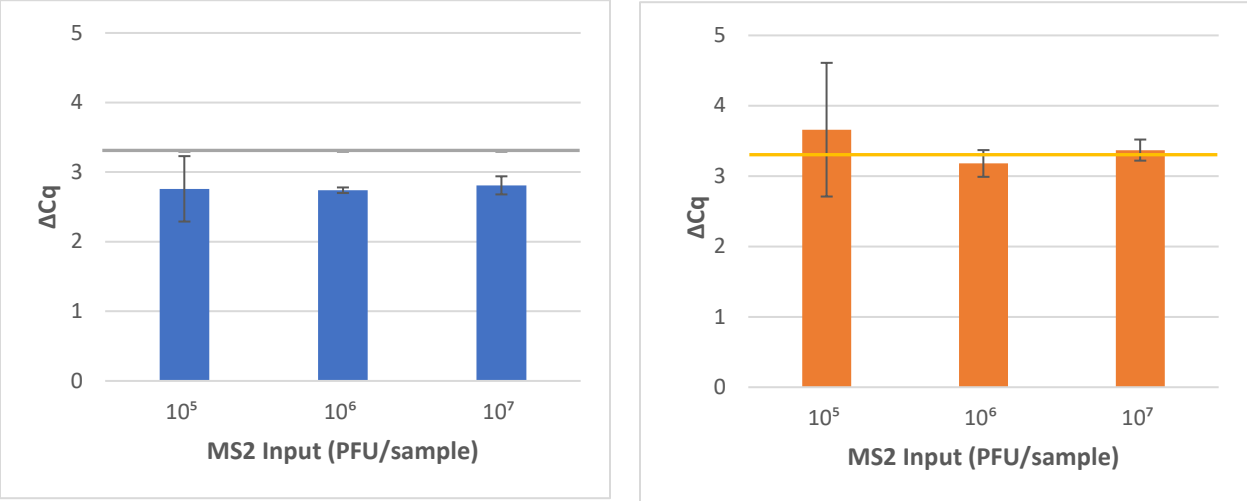

Figure 2. Average ΔCq from amplification of undiluted and 1:10 diluted eluates from purification of 10mg of pig liver (left) or scallop (right) spiked with MS2 bacteriophage. Undiluted and 1:10 diluted eluates were amplified via RT-qPCR with MS2-specific primers/probes. Assuming 100% PCR efficiency, a ΔCq of 3.3 indicates no qPCR inhibition (indicated by the grey and yellow lines). No significant qPCR inhibition was observed in undiluted scallop sample eluates. Slight inhibition may be present in undiluted liver eluates, which may be due to their high concentration of total RNA (average of 451ng/µl). Data are represented as mean ± standard deviation for n=3. ΔCq values for MS2 input of 10^4 PFU/sample are not shown as the Cqs of 1:10 dilutions were >40 or some replicates did not amplify.