Promega

Product Application

Automated Purification of Viral RNA from Pork Sausage

Purify viral RNA from pork sausage using the Maxwell® RSC PureFood GMO and Authentication Kit.

Kit: Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)

Analyses: RT-qPCR

Sample Type(s): Pork sausage

Input: 10mg

Materials Required:

- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Maxwell® RSC Instrument (Cat.# AS4500)
- 1-Thioglycerol (Cat.# A208B)
- Heat block
- Tissue homogenizer

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 TM473, available at: **www.promega.com/protocols**

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

Protocol:

1. Prepare volume of CTAB Buffer + 1-Thioglycerol for the number of samples (add 20µl of 1-Thioglycerol for every 1ml of CTAB Buffer).
2. Homogenize 10mg sample in 600µl CTAB Buffer + 1-Thioglycerol.
3. Add 30µl Proteinase K to sample, invert and vortex to mix. Do not add RNase to sample.
4. Place sample in heat block at 60°C for 30 minutes.
5. Prepare the Maxwell® RSC PureFood GMO and Authentication Kit cartridge as follows:
    a. Place plunger in well #8 of the cartridge.
    b. Place elution tube with 100µl Elution Buffer in the deck tray.
    c. Add 30µl Lysis Buffer to well #1 of the cartridge.
6. Remove sample from heat, vortex to mix.
7. Spin sample at ≥ 16,000 x *g* for 10 minutes at room temperature.
8. Transfer 300µl cleared lysate to well #1 of the cartridge.
9. Load the deck tray onto the Maxwell® RSC Instrument.
10. Select and run the PureFood GMO and Authentication method.
11. Store purified RNA at -70°C.

Results: Linear detection of MS2 bacteriophage was observed across a 4-log range. No qPCR inhibition was observed in eluates.

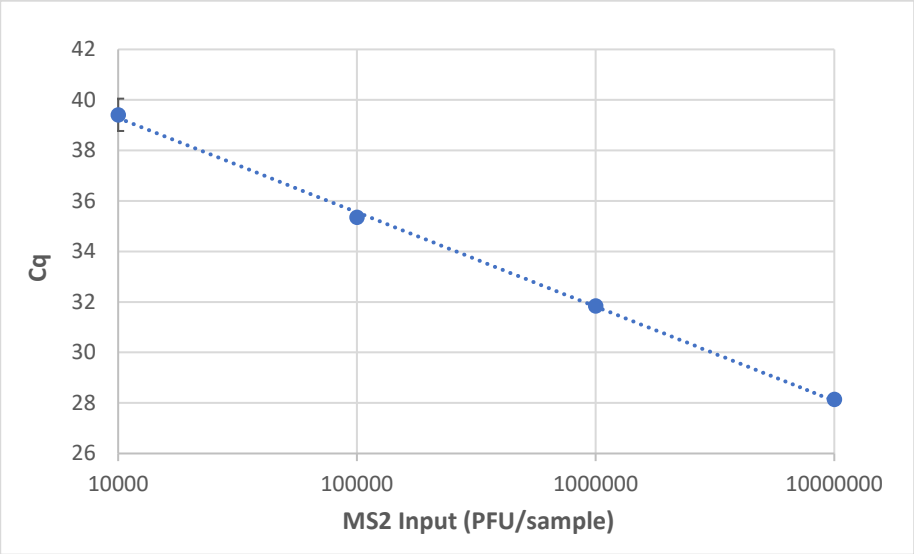

Figure 1. Average Cq values from 10mg of pork sausage samples spiked with MS2 bacteriphage across a 4-log range. Samples were purified using the Maxwell® RSC PureFood GMO and Authentication Kit and amplified by RT-qPCR using the GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6120) with MS2-specific primers/probes. Samples showed linear detection across a 4-log range. Data are represented as mean ± standard deviation for n=3 replicates.

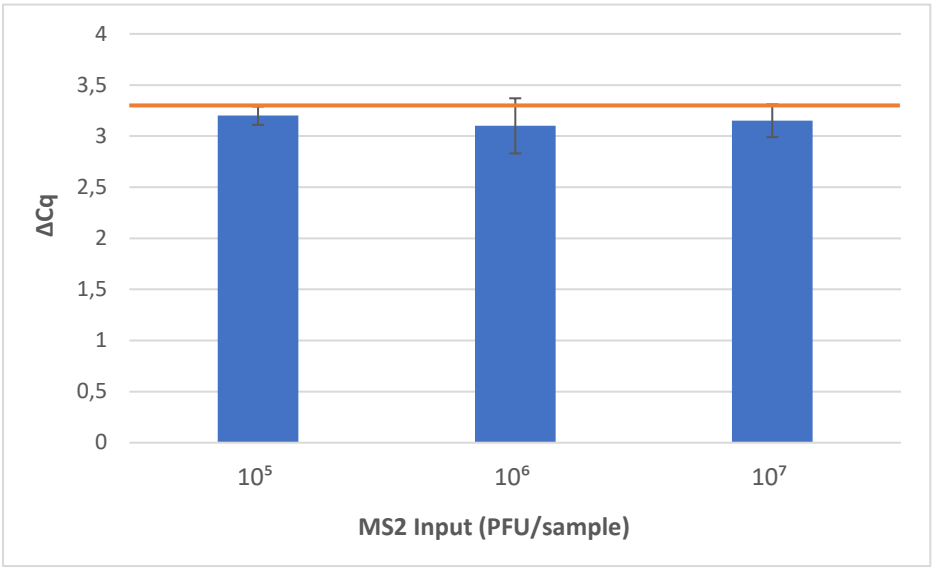

Figure 2. Average ΔCq from amplification of undiluted and 1:10 diluted eluates from purification of 10mg pork sausage spiked with MS2 bacteriophage sample. Undiluted and 1:10 diluted eluates were amplified by RT-qPCR using the GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6120) with MS2-specific primers/probes. Assuming 100% PCR efficiency, a ΔCq of 3.3 indicates no qPCR inhibition (indicated by orange line). No qPCR inhibition was seen in undiluted eluates. Data are represented as mean ± standard deviation for n=3 replicates. ΔCq value for MS2 input of 10^4 PFU/sample is not shown as the Cqs of 1:10 dilutions were >40 or some replicates did not amplify.