Promega

Product Application

Automatic purification of viral DNA from raw and processed food products containing pork

Purify viral DNA from raw and processed pork products using the Maxwell® RSC Instrument and Maxwell® RSC Tissue DNA kit

Kit: Maxwell® RSC Tissue DNA kit (Cat.# AS1610)

Analyses: qPCR

Sample Type(s): Raw pork shoulder meat and processed pork products (pork jerky and Chinese sausage)

Input: 100mg of pork sample

Materials Required:

- Maxwell® RSC Tissue DNA Kit (Cat.# AS1610)
- Tissue Lysis Buffer (Cat.# A5091)
- Proteinase K solution (Cat.# MC5008)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Heat Block (set at 70°C)
- Microcentrifuge

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 TM476, available at: **www.promega.com/protocols**

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

Protocol:

1. Add 400µl of TLA buffer and 35µl of Proteinase K to each 1.5ml tube containing 100mg of pork sample.
2. Vortex vigorously on maximum speed for 10 seconds to mix.
3. Place samples in a standard heat block at 70°C for 60 minutes.
4. Centrifuge for 10 minutes at 16,000 *x g* to separate any solid or oils.
5. Transfer the cleared sample supernatant into well #1 of the Maxwell® RSC cartridge. Avoid pipetting any solid material from the bottom of the tube or oil from the surface.
6. Add plungers to well #8 of the Maxwell® RSC cartridge.
7. Place the supplied elution tubes into the sample rack and add 100µl of the elution buffer.
8. Run the method *Maxwell® RSC Tissue DNA kit* on the Maxwell® RSC Instrument.

Results:

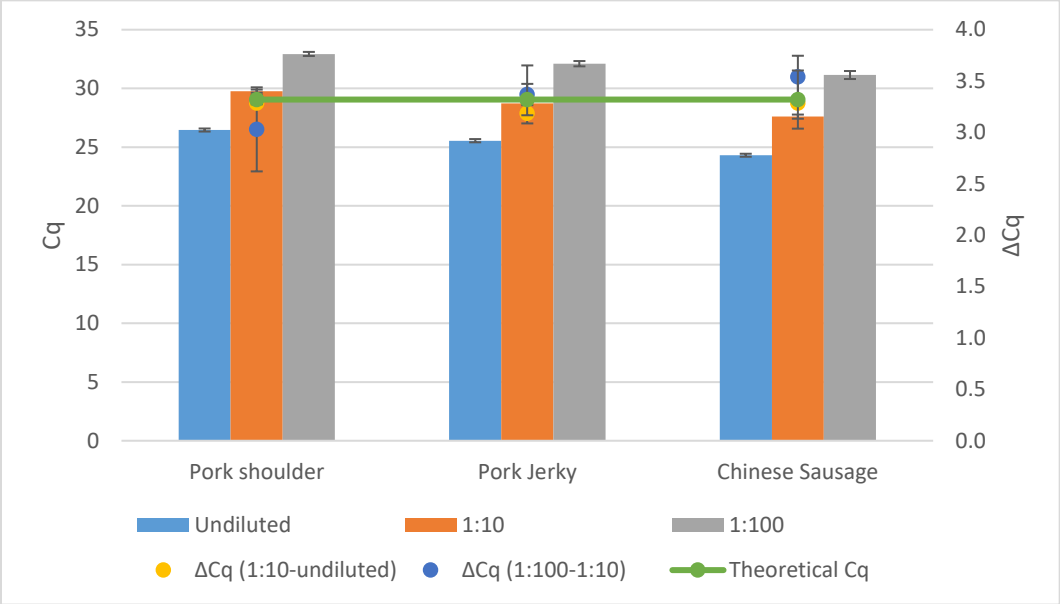

Figure 1. qPCR amplification of DNA isolated from different pork products spiked with Lambda phage. $5x10^5$ PFU of lambda phage was diluted at a ratio of 1:10 or 1:100 and spiked into three different pork containing samples. DNA was isolated using the protocol described in this Application Note. Viral DNA was detected using GoTaq® probe qPCR system (Cat.# A6101) with lambda phage specific primers and probe. Cq values of lambda viral DNA recovered from different pork containing products was shown. There is approximately a 3.3 Cq difference between the undiluted and diluted virus spiked samples, indicating the purification efficiency was linear. Undiluted DNA eluate prepared from 100mg raw pork shoulder was diluted at 1:10 and amplified with lambda phage specific primers and probe to examine the amplification efficiency. Average values from three experiments ± STD are shown.