Promega

Product Application

RNA extraction from mussel to detect viral RNA following the ISO 15216-2 method

RNA extraction from mussels spiked with Mengo virus (Mengo Extraction Control CeeramTOOLS®) using the Maxwell® RSC PureFood GMO and Authentication Kit on the Maxwell® RSC Instrument, using preprocessing described in ISO 15216-2 method (1). Percent of recovery determined by RT-qPCR using the GoTaq® Probe 1-Step RT-qPCR System.

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

GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6120)

Analyses: Probe-based RT-qPCR

Sample Type(s): Mussel

Input: 2g of digestive gland

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.

Further information can be found by e- mailing Technical Services techserv@promega.com

Materials Required:

- Mengo Extraction Control CeeramTOOLS® (Biomérieux, Ref. KMG).
- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC PureFood GMO and Authentication kit (Cat.# AS1600)
- razor blade
- incubator and water bath

Protocol:

1. **RNA extraction using the Maxwell® RSC PureFood GMO and Authentication Kit**

Preprocessing samples for Bivalve molluscan shellfish (BMS) from the ISO 15216-2 method.

- Finely chop 2g of mussel digestive gland using a razor blade and transfer to a 15ml tube.
- Add 10µl of Mengo Extraction Control CeeramTOOLS®.
- Add 2ml of proteinase K solution (2ml of water + 10µl of proteinase K, 20mg/ml). Vortex.
- Incubate at 37°C for 1 hour, under shaking at 300rpm/min.
- Incubate at 60°C for 15 minutes in a water bath.
- Centrifuge at 3,000 × *g* for 5 minutes.
- Decant all supernatant into a clean 15ml tube. Measure and record the volume (ml) and retain for RNA extraction.

RNA extraction using the Maxwell® RSC PureFood GMO and Authentication Kit

- Transfer 500µl of supernatant to a clean 1.5ml tube (**Note:** Extra 1.5ml tubes containing 500µl of Nuclease-Free Water were used as a negative extraction control of the Maxwell® RSC extraction method).
- Add 400µl of CTAB.

- Add 30µl of Proteinase K (PK) Solution (**Note:** Avoid addition of RNase A Solution). Vortex.
- Incubate at 60°C for 30 minutes with shaking at 600rpm. Vortex.
- Centrifuge for 10 minutes at room temperature at ≥16,000 × *g* to separate any oils and solids.
- Add 300µl of Lysis Buffer to well #1 of the cartridge.
- Add all lysate sample to well #1.
- Place elution tubes, and add 100µl of Elution Buffer.
- Place the plunger into well #8 of each cartridge.
- Place the cartridge rack into the Maxwell® RSC Instrument, and run the Maxwell® RSC PureFood GMO and Authentication Kit protocol (for more information see technical manual #TM473).
- Retain extracted RNA for RT-qPCR amplification (**Note:** Extracted RNA shall be processed immediately or stored at (5±3)°C for < 8h or at –15°C or below for up to 6 months).

Results:

Mussels spiked with Mengo Extraction control CeeramTOOLS®, as described in the preprocessing on the ISO 15216-2 method, were extracted using a modified preprocessing method described above for the Maxwell® RSC PureFood GMO and Authentication Kit on the Maxwell® RSC Instrument. Mengo virus recovery was determined by RT-qPCR using specific probe and primers (1) and the GoTaq® Probe 1-Step RT-qPCR System (ISO requirement for extraction method: >1% of recovery) (Figure 1).

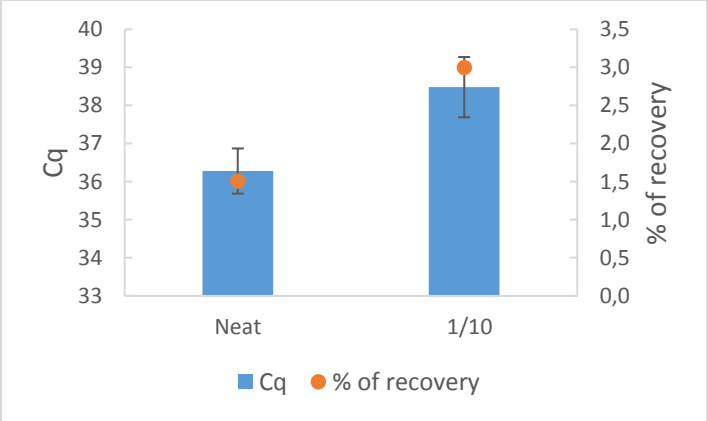

Figure 1. Mengo virus recovery from spiked mussels using the Maxwell® RSC PureFood GMO and Authentication Kit with the Maxwell® RSC Instrument. Cq values and % of recovery determined by RT-qPCR to detect Mengo virus RNA using specific probe primers (1) and the GoTaq® Probe 1-Step RT-qPCR System (efficiency of reaction: 103%, r2: 0.98, slope –3.23). Negative extraction controls were undetermined, showing no cross-contamination during the RNA extraction process. NTC amplifications were undetermined (data not shown) (mean ± STD for n=3).
For more information related to the viral Mengo virus RNA detection, see Viral RNA detection using the GoTaq® Probe 1-Step RT-qPCR System following the ISO 15216-2 method- Application Note (2).

References:

1. *ISO 15216-2 method.*
2. *Viral RNA detection using the GoTaq® Probe 1-Step RT-qPCR System following the ISO 15216-2 method* – Application Note.