

Product Application

Automatic purification of viral Total Nucleic Acid from Cow Milk

Purify viral RNA and DNA from cow milk that is detectable at low concentrations using Maxwell® RSC instrument and Maxwell® RSC PureFood GMO and Authentication Kit

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

Analyses: qPCR and RT-qPCR

Sample Type(s): Cow milk (approx. 3.5% milkfat) spiked with MS2

and Lambda bacteriophages

Input: 200μl milk with DNA and RNA bacteriophage

Materials required:

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

Maxwell® RSC instrument (Cat.# AS4500)

Heat block (set at 56°C)

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. Add 200μl of milk samples to 1.5ml tube.
- 2. Add 300µl of Lysis Buffer and 30µl of Proteinase K. Vortex for 15 seconds.
- 3. Incubate at 56°C for 20 minutes.
- 4. Add entire lysate into well #1 of the Maxwell® RSC cartridges.
- 5. Omit the addition of RNase to the cartridge.
- 6. Elute in 100μl of Elution Buffer.



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Results:

Table 1. Titrations of viral RNA (MS2) and viral DNA (Lambda) into cow milk purified with the Maxwell® RSC PureFood GMO and Authentication Kit.

	MS2 (PFU/μl)	Lambda (PFU/μl)
Titration 1	10 ⁷	10^{6}
Titration 2	10 ⁶	10 ⁵
Titration 3	10 ⁵	10 ⁴
Titration 4	10 ⁴	10 ³

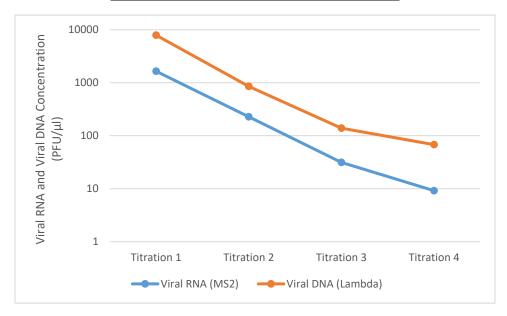


Figure 1. Viral RNA and viral DNA concentrations following titrations of MS2 and Lambda bacteriophages into cow milk. MS2 RNA virus and Lambda DNA virus were titrated into cow milk and purified in triplicate simultaneously with the Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600). Amplification with primers to MS2 bacteriophage using GoTaq® probe 1-Step RT-qPCR (Cat.# 6120) and primers to Lambda bacteriophage using GoTaq® Probe qPCR system (Cat.# A6101) was performed. The concentration of viral spike-in that was reliably detected after purification from a 200µl sample with the Maxwell® RSC PureFood GMO and Authentication kit (Cat.# AS1600) was 10⁵ PFU/200µl for MS2 and 10³ PFU/200µl with Lambda.