Promega

Product Application

Automated Purification of Viral Total Nucleic Acid from Human Breast Milk

Purify viral RNA and viral DNA from human breast milk. Nucleic acid 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): Human breast milk

Input: 200µl

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 by e-mailing 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 Solution. 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.
7. Run the Maxwell® RSC PureFood GMO and Authentication method on the Maxwell® RSC Instrument.

Results:

Table 1. Titrations of viral RNA (MS2) and viral DNA (Lambda) into human breast 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

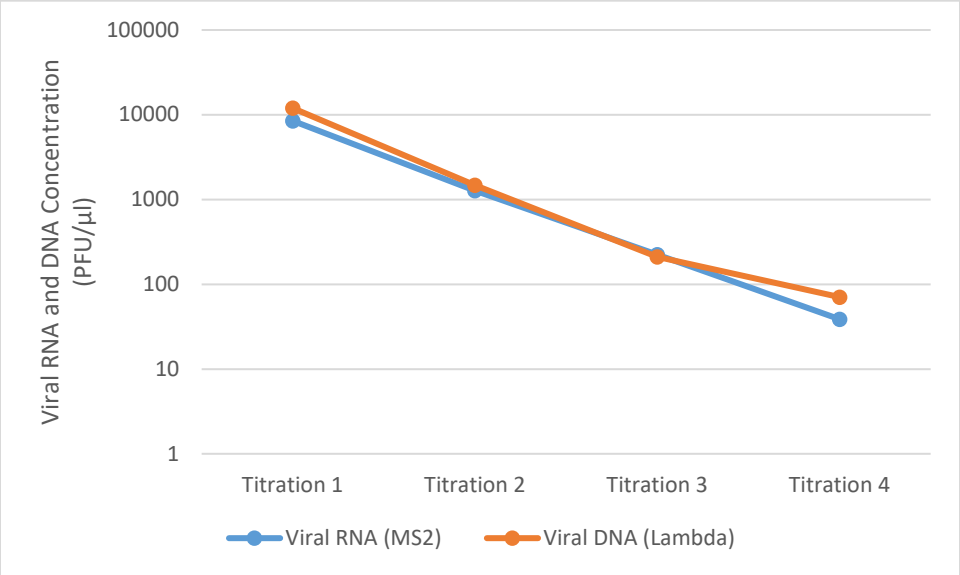

Figure 1. Viral RNA and viral DNA concentrations following titrations of MS2 and Lambda bacteriophages into human breast milk. MS2 RNA virus and Lambda DNA virus were titrated into human breast milk and purified in triplicate simultaneously with the Maxwell® RSC PureFood GMO and Authentication Kit on the Maxwell® RSC Instrument. Amplification with primers to MS2 bacteriophage using GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6120) 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 was 10^5 PFU/200µl for MS2 and 10^3 PFU/200µl with Lambda.