

Purification of Pathogen Nucleic Acid from Pig Oral Fluid Using the Maxwell® RSC Instrument

Isolate total nucleic acid from pig oral fluid using the using the Maxwell® RSC Instrument and Maxwell® RSC PureFood GMO and Authentication Kit.

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

Analyses: RT-qPCR or qPCR for specific detection of spiked pathogens

Sample Type(s): Pig oral fluid from chew ropes

Input: 200µl

Materials Required:

- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Maxwell® RSC instrument (Cat.# AS4500)
- 1-Thioglycerol (Cat.# A208B)

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 CTAB + 2% 1-Thioglycerol (100µl needed per sample; 20µl of 1-thioglycerol in 1ml of CTAB).
2. To 200µl of pig oral fluid, add 100µl of CTAB + 2% 1-Thioglycerol. Incubate sample at room temperature for 10 minutes.
3. Add entire sample and 300µl of Lysis Buffer to well #1 of the Maxwell® RSC cartridge. Place a plunger in well #8 of the Maxwell®, and add 50µl of Elution Buffer to the bottom of the provided Elution Tube.
4. Process samples with the PureFood GMO and Authentication protocol on the Maxwell® RSC Instrument.

Results:

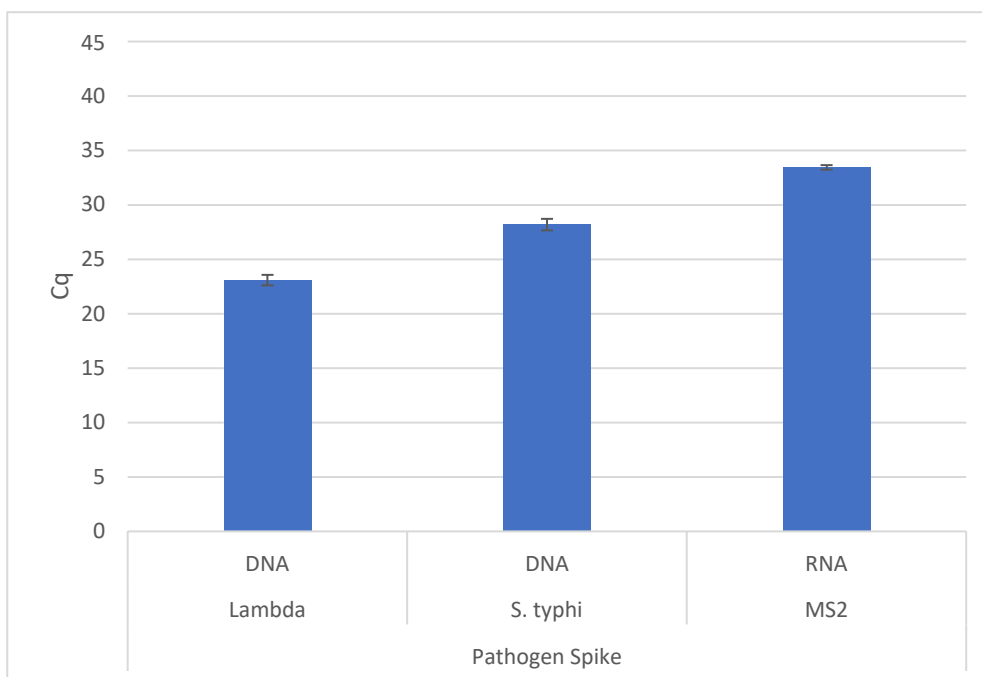


Figure 1. Detection of mock pathogen nucleic acid spiked into pig oral fluid. Pig oral fluid was spiked with MS2 bacteriophage (RNA viral model), Lambda bacteriophage (DNA viral model), or *Salmonella typhi* (*S. typhi*; Gram-negative bacteria). Samples were purified according to the method described above using the Maxwell® RSC PureFood GMO and Authentication Kit on the Maxwell® RSC Instrument. The presence of the mock pathogen nucleic acid was detected in 2µl of eluate by RT-qPCR (for MS2 RNA) with the GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6121) and by qPCR in 2µl of eluate (for Lambda DNA) or in 5µl of eluate (for *S. typhi* DNA) with the GoTaq® Probe qPCR System (Cat.# A6101) in 20µl reactions containing mock pathogen-specific primers/probe according to the respective technical manuals. Data represent the average of quadruplicate purifications and duplicate amplifications.