Promega

Product Application

Viral TNA extraction from Cerebrospinal Fluid (CSF) using ReliaPrep™ Viral TNA MiniPrep, Custom Purification Kit

Purification of total nucleic acid (TNA) for pathogen testing (viral DNA, viral RNA and bacterial DNA) from cerebrospinal fluid (CSF) using the ReliaPrep™ Viral Total Nucleic Acid Purification Kit.

Kit: ReliaPrep™ Viral TNA MiniPrep, Custom (Cat. #AX4820)

Analyses: qPCR amplification with GoTaq® Probe Master Mix (Cat. #A6102) and 1-step RT-qPCR amplification with GoTaq® Probe 1-step RT-qPCR System (Cat. #A6120)

Sample Type(s): Cerebrospinal Fluid (CSF)

Input : 200µl

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 at techserv@promega.com.

Materials Required:

- ReliaPrep™ Viral TNA MiniPrep, Custom (Cat. #AX4820)
- Microcentrifuge
- Heat block
- Vortex mixer

Protocol:

1. Dispense 20µl of Proteinase K (PK) Solution into a 1.5ml microcentrifuge tube.
2. Thoroughly mix CSF, add 200µl to the tube containing the PK Solution. Briefly mix.
3. Add 200µl of Cell Lysis Buffer to the tube. Cap and vortex for at least 10 seconds.
4. Incubate samples at 56°C for 10 minutes.
5. While the sample is incubating, place a ReliaPrep™ Binding Column into an empty Collection Tube.
6. Remove tube from heating block. Add 250µl of Binding Buffer, cap the tube and mix by vortexing for 10 seconds.
7. Add the sample tube contents to the ReliaPrep™ Binding Column, cap and centrifuge for 1 minute at maximum speed. Ensure all lysate has completely passed through the membrane. Centrifuge for another minute to remove any remaining lysate.
8. Place binding column into a fresh collection tube.
9. Add 500µl of Column Wash Solution to the column and centrifuge for 3 minutes at maximum speed. Discard flow through.
10. Repeat step 9 twice for a total of 3 washes.
11. Place the ReliaPrep™ Binding Column in a clean 1.5ml microcentrifuge tube.
12. Add 50-200µl of Nuclease-Free Water to the column. Centrifuge 1 minute at maximum speed.
13. Discard the ReliaPrep™ Binding Column and save the eluate.

Results:

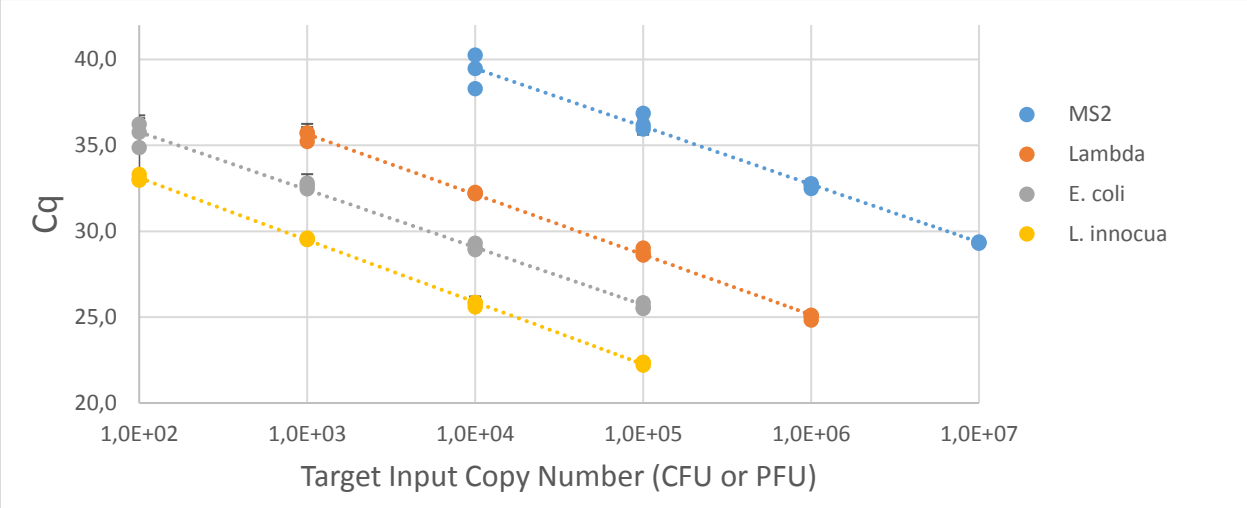

Figure 1. Average Cq values from CSF spiked with Lambda (DNA)/MS2 (RNA) viruses or *E. coli* and *L. innocua* (DNA) bacteria across a 4-log range extracted using ReliaPrep™ Viral TNA MiniPrep, Custom Kit. Samples were amplified with pathogen specific primers/probes. All targets showed linear detection across a 4-log range. Data represented as the mean ± standard deviation for n = 3.

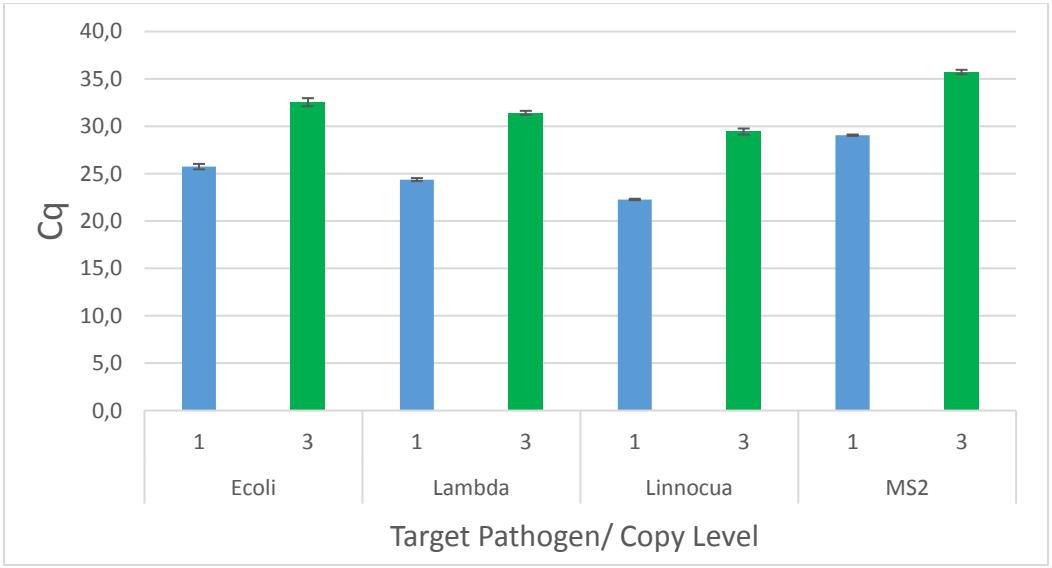

Figure 2. Average Cq values amplified from CSF spiked with all four pathogen targets (Lambda (DNA) bacteriophage, MS2 (RNA) bacteriophage, *E. coli* and *L. innocua* (DNA) bacteria) extracted using ReliaPrep™ Viral TNA MiniPrep, Custom Kit. Level 1 = $1x10^5$ CFU of *E. coli* and *L. innocua*, $1x10^6$ PFU Lambda bacteriophage and $1x10^7$ PFU MS2 bacteriophage. Level 3 = $1x10^3$ CFU of *E. coli* and *L. innocua*, $1x10^4$ PFU Lambda bacteriophage and $1x10^5$ MS2 bacteriophage. Samples were amplified with pathogen specific primers/probes. Data represented as the mean ± standard deviation for n = 3. All targets were detectable from a single CSF sample at both copy levels tested.