Promega

Product Application

Viral Nucleic Acid Isolation from Cell Culture Media

To isolate total nucleic acids from viruses in cell culture media using ReliaPrep™ Blood gDNA Miniprep System.

Kit: ReliaPrep™ Blood gDNA Miniprep System

Analyses: qPCR or RT-qPCR

Sample Type(s): Cell Culture Media

Input: 200µl of Sample

Materials Required:

- Heat Block capable of 56°C

This protocol was developed by Promega Applications Scientists and is intended for research use only.

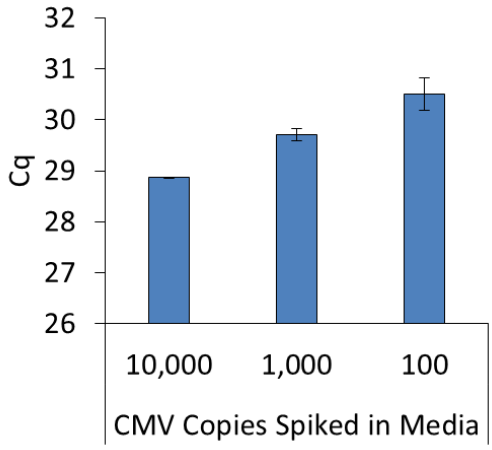
Users are responsible for determining suitability of the protocol for their application.

Further information can be found in Technical Manual #TM330, available at: **www.promega.com/protocols** or for further information, please contact **techserv@promega.com**

Protocol:

1. Add 20µl proteinase K to 200µl of cell culture media sample and vortex.
2. Add 200µl CLD to the sample. Mix by vortexing for at least 10 seconds.
3. Incubate 56°C for 10 minutes.
4. Add 250µl of BBA buffer and vortex to mix.
5. Load lysate to binding column/collection tube assembly.
6. Centrifuge at max speed for 1 minute.
7. Add 500µl CWD and centrifuge at max speed for 3 minutes. Discard flow through.
8. Repeat step 7.
9. Place column in a clean Elution Tube. Add 50µl of Nuclease-Free Water to the column.
10. Centrifuge at max speed for 1 minute. Store eluted nucleic acid at -20°C or -70°C.

Results: Cytomegalovirus (CMV) DNA Isolation from Cell Culture Media



Cq values from RT-qPCR for Zika virus with different sample types. Nucleic acid was purified from cell culture media spiked with NATtrol® CMV (Zeptometrix, Cat. #NATCMV-LIN) at 10,000, 1,000, and 100 copies per 200µl of media. Viral DNA was detected by amplification with GoTaq® qPCR Master Mix (A60601) using CMV specific primers. n=3 for each condition.

