

Viral Nucleic Acid Isolation from Plasma

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

Kit: ReliaPrep™ Blood gDNA Miniprep System

Analyses: RT-qPCR or qPCR

Sample Type(s): Plasma

Input: 200µl of Sample

Materials Required:

- Heat Block capable of 56°C

Protocol:

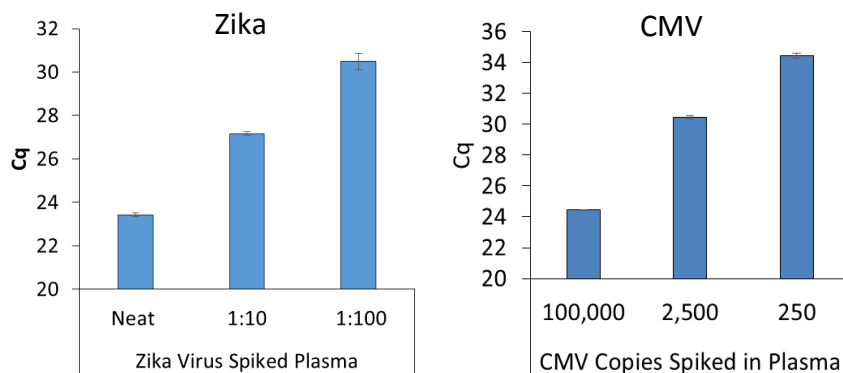
1. Add 20µl proteinase K to 200µl of plasma in a 1.5ml tube and vortex.
2. Add 200µl CLD to the tube. Mix by vortexing for at least 10 seconds.
3. Incubate 56°C for 10 minutes.
4. Add 250µl of BBA buffer and vortex to mix.
5. Load lysate to binding column/collection tube assembly.
6. Centrifuge at max 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 acids at -20°C or -70°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

Results with spiked RNA and DNA Viruses in Plasma



Cq values from RT-qPCR for Zika Virus with RNA from Plasma (Left). Zika Virus obtained from Zeptomatrix was used for testing (Zeptomatrix, Cat. # NATZIKV-ERCM). Zika Virus was spiked into human plasma at a ratio of 1:10 or 1:100 v/v. Viral recovery was tested by amplification with GoTaq® 1-step RT-qPCR System (A6020) using Zika Virus specific primers. n=3 for each condition.

Cq values from qPCR for HCMV with DNA from Plasma (Right). Nucleic acid was purified from plasma spiked with CMV (Zeptomatrix, Cat. #NATCMV-LIN) at 100,000, 2,500, or 250 copies per 200µl of plasma. Viral DNA was detected by amplification with GoTaq® qPCR Master Mix (A6001) using CMV specific primers. n=3 for each condition.