Promega

Product Application

High-Throughput purification of Viral Total Nucleic Acid from Plasma on the Hamilton STAR Automated Liquid Handler

Purify both DNA and RNA from viruses in plasma using the Maxwell® HT Viral Total Nucleic Acid Kit on the Hamilton STAR Automated Liquid Handler.

Kit: Maxwell® HT Viral Total Nucleic Acid Kit (Cat.# AX2340)

Analyses: qPCR and RT-qPCR

Sample Type(s): MS2 and Zika RNA virus in plasma; Lambda and HBV DNA virus in plasma

Input: 100–300µl plasma

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, contact Technical Services at: **techserv@promega.com**

Instrument Requirements:

- Hamilton STAR Automated Liquid Handler
- 8 independent pipetting channels
- 96 CO-RE head
- 2 Hamilton Heater Shakers (HHS)
- Nunc® 2.0ml Deep-Well Plate Adapter
- Carriers for SBS plates, tip racks, 60ml and 200ml reagent reservoirs
- MagnaBot® Flex Separation Device (Cat.# AX5600)

Consumables Required:

- four 60ml Reagent Troughs (reusable between runs) (Cat.# AS9304)
- four 200ml Reagent Troughs (reusable between runs) (Hamilton Cat.# 56695-01)
- 1000µl CO-RE Tips (Cat.# AS9303)
- Standard Volume (300µl) CO-RE Tips (Cat.# AS9302)
- four Nunc® 2.0ml Deep Well Plates (Cat.# AS9307)

Protocol:

The procedure described below has been modified for automation of the Maxwell® HT Viral TNA Kit on the Hamilton STAR in a 96-well plate format.

1. Add isopropanol to 4/40 Wash Solution, as indicated on the bottle.
2. Add ethanol and isopropanol to Alcohol Wash, Blood, as indicated on the bottle.
3. Prepare 80% ethanol.
4. Add 100–300µl of plasma sample to a Nunc® 2.0ml Deep Well Processing Plate.
5. The method uses the reagents and volumes per well shown in Table 1.

Table 1. Volumes of Reagents Used in the Maxwell® HT Viral TNA method on the Hamilton STAR.

Reagents	Volume per well (µl)
Lysis Buffer	1:1 volume with sample volume (100–300µl)
Proteinase K (PK) Solution	1:10 volume with sample volume (10–30µl)
Isopropanol	700
MagneSil® RED	50
4/40 Wash Solution	225
Alcohol Wash, Blood	200
80% Ethanol	200
Nuclease-Free Water	50–110

6. Launch the HamiltonSTAR_ViralTNA_v1.0 method in the Hamilton Run Controller.
7. Set up the instrument deck as shown in Figure 1.
8. In the Prompt, select the number of samples to be run, the desired sample input volume and elution volume.
9. Add the reagents to the appropriate reagent reservoirs, as indicated in the Run Setup Screens, and then acknowledge the prompt by clicking ok to continue the run.

Summary of the main steps of the Maxwell® HT Viral TNA on the Hamilton STAR method:

- Sample lysis with Lysis Buffer and Proteinase K during a heated incubation.
- Addition of isopropanol (binding buffer) and sample cooling.
- Nucleic acid captured on resin with vigorous shaking and tip mixing.
- Removal of lysate from resin.
- Resin washed three times with 4/40 Wash, Alcohol Wash and 80% Ethanol Solution.
- Resin dried thoroughly with shaking but no heat.
- Elution with nuclease-free water using vigorous shaking and tip mixing.
- Eluate transferred from the processing plate to a clean elution plate.

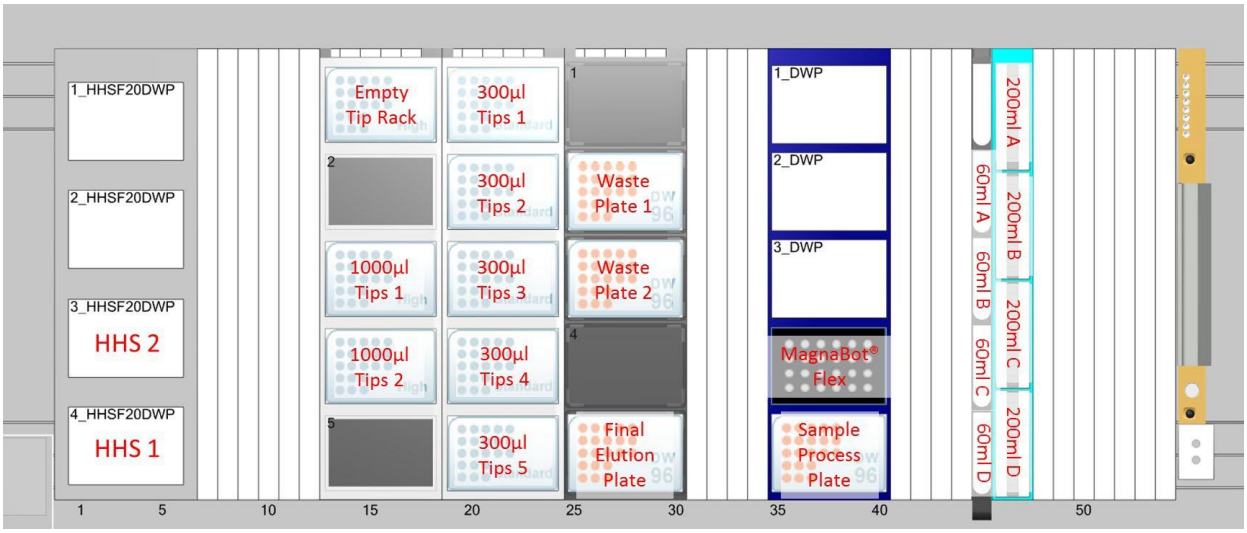

Figure 1. Deck layout for Maxwell® HT Viral TNA on the Hamilton STAR Automated Liquid Handler. The deck layout is described from left to right. HHS 1 is Hamilton Heater Shaker with a plate adapter for heat transfer to the Nunc® 2.0ml Deep Well Plate. HHS 2 is a second Hamilton Heater Shaker, which does not require a plate adapter because it is used only for sample mixing, not for sample heating. In the tip carrier at deck position 13-18, position 1 contains an Empty Tip Rack used for reracking tips between resin mix steps, and positions 3 and 4 contain 1000µl tips used by the pipetting channels. In the tip carrier at deck position 19-25, positions 1–5 contain 300µl tips used by the 96 CO-RE head; in these tip racks, only place as many tips as the number of samples being processed. The tip rack placed at position 1 should be replaced before each run because tips are reracked here during pipette mixing steps of the binding protocol. On a plate carrier at deck position 25-30 place empty Nunc® 2.0ml Deep Well Plates at positions 2, 3 and 5. On a low-profile plate carrier at deck position 35-40 place the MagnaBot® Flex at position 4 and the Sample Processing Plate containing the samples to be processed at position 5. In a 60ml trough carrier at position 45, reagent reservoir A is loaded with Resin, reagent reservoir B is loaded with Lysis Buffer, reagent reservoir C is loaded with Proteinase K and reagent reservoir D is loaded Nuclease-Free Water. In a 200ml trough carrier at deck position 46-47, reagent reservoir A is loaded with Isopropanol (Binding Buffer), reagent reservoir B is loaded with 4/40 Wash, reagent reservoir C is loaded with Alcohol Wash and reagent reservoir D is loaded with 80% Ethanol. The volumes of each reagent to be loaded onto the instrument are described in the method setup screens.

Results:

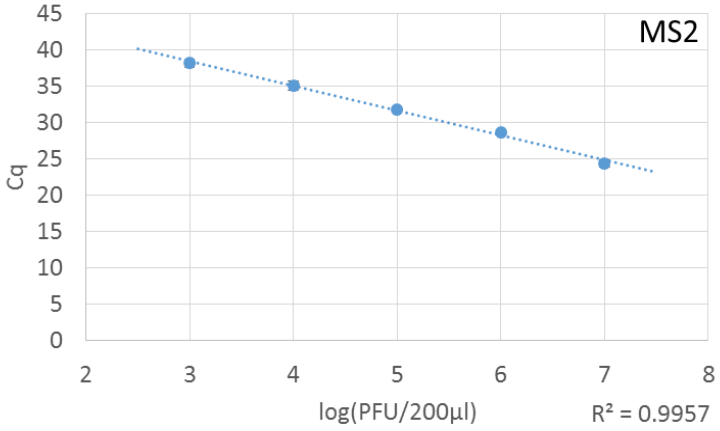

Figure 2. Linearity of nucleic acid recovery over a 5-log range of MS2 spiked into plasma samples. A set of plasma samples spiked with MS2 ranging from 10^7PFU/200µl sample down to 10^3PFU/200µl sample was purified in quadruplicate. Cq values were determined using the GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6120) and MS2 primer/probe set.

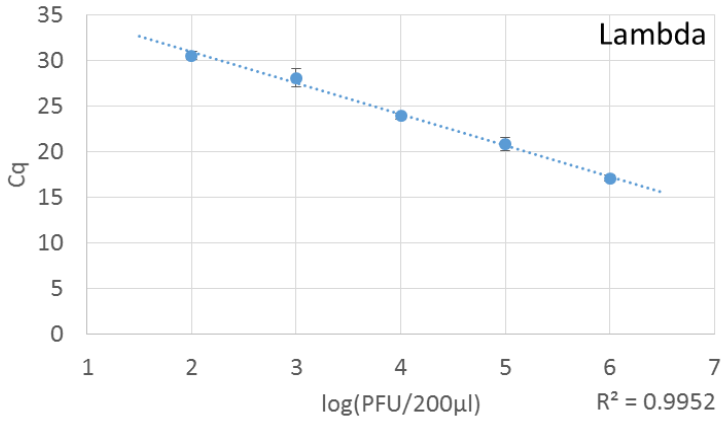

Figure 3. Linearity of nucleic acid recovery over a 5-log range of Lambda spiked into plasma samples. A set of plasma samples spiked with Lambda ranging from 10^6PFU/200µl sample down to 10^2PFU/200µl sample was purified in quadruplicate. Cq values were determined using the GoTaq® Probe qPCR Master Mix (Cat.# A6102) with Lambda primer/probe set.

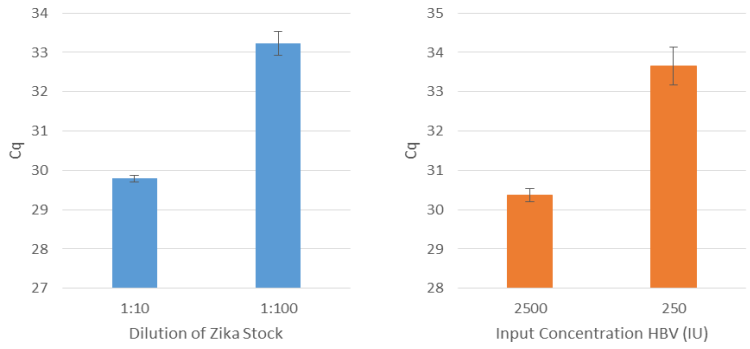

Figure 4. Nucleic acid recovery from Zika (RNA virus) and HBV (DNA virus) spiked into plasma. Zika (left panel) was spiked into human plasma at 1:10 and 1:100 dilutions of the provided stock. HBV (right panel) was spiked into human plasma at a concentration of 2500 IU/200µl sample and diluted 1:10 for a final concentration of 250IU/200µl sample. Samples were purified in quadruplicate, and recovered nucleic acid was detected in RT-qPCR using the GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6120) and a Zika primer/probe set or using the GoTaq® Probe qPCR Master Mix (Cat.# A6102) with an HBV primer/probe set.

	1	2	3	4	5	6
A	ND	28.8	ND	28.2	ND	28.3
B	27.9	ND	28.7	ND	28.3	ND
C	ND	28.9	ND	28.2	ND	28.4
D	28.9	ND	28.9	ND	28.2	ND*
E	ND	28.9	ND	27.9	ND	28.7
F	28.7	ND	29.2	ND	28.4	ND*
G	ND	28.6	ND	28.3	ND	28.4
H	28.9	ND	28.8	ND	28.1	ND*

Figure 5. Cross-contamination study. Plasma samples were spiked with MS2 at 10^6PFU/200µl sample. Two hundred microliters of MS2-spiked or unspiked plasma samples were added to a deep-well plate in a checkerboard pattern across the first 6 columns of wells. Average Cq values of triplicate amplification in RT-qPCR with the GoTaq® Probe 1-Step RT-qPCR System are given for each well. For all wells containing MS2, each of the triplicate amplification performed for each well was positive for MS2 with an average Cq value of 28.5 ± 0.7. Samples denoted with * showed amplification in 1/3 replicates. Samples were not considered positive for cross-contamination unless 2/3 amplification replicates were positive for MS2 detection. Thus, no control samples were flagged for cross-contamination.

References:

1. Lanciotti *et al*. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007.
2. Sun, S. *et al*. Development of a new duplex real-time polymerase chain reaction assay for Hepatitis B viral DNA detection. *Virol J.*, 2011.