Promega

Product Application

High Throughput Purification of Viral Total Nucleic Acid from Plasma on the Tecan Freedom EVO 150 Automated Liquid Handler

Efficient purification of DNA and RNA from viruses in plasma using the Maxwell® HT Viral Total Nucleic Acid Kit on the Tecan Freedom EVO 150 Automated Liquid Handler.

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

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

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

Input: 50–300µl 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.

Further information can be found by e-mailing technical services at techserv@promega.com

Instrument Requirements:

- Tecan Freedom EVO 150 Automated Liquid Handler
- 8 channel LiHa
- 96 MCA head
- QInstruments Bioshake D30-T elm
- Nunc® 2.0ml Deep-Well Plate Adapter
- Carriers for SBS plates, tip racks, 25ml and 100ml reagent reservoirs
- MagnaBot® Flex Separation Device (Cat.# AX5600)

Consumables Required:

- 3 – 25ml Reagent Troughs (re-usable between runs) (Tecan Cat.# 30055743)
- 5 – 100ml Reagent Troughs (re-useable between runs) (Tecan Cat.# 10613049)
- 1000µl LiHa Filtered Disposable Tips (Tecan Cat.# 30126020)
- 200µl LiHa Filtered Disposable Tips (Tecan Cat.# 30126018)
- 200µl MCA Filtered Disposable Tips (Tecan Cat.# 30038618)
- 2 – 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 Tecan Freedom EVO 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 following reagents and volumes per well (Table 1):

Reagents	<200µl Sample Volume per well (µl)	≥200µl Sample Volume per well (µl)
Lysis Buffer	200	1:1 volume with sample volume
Proteinase K (PK) Solution	20	1:10 volume with sample volume
Isopropanol	700	700
MagneSil® RED	50	50
4/40 Wash Solution	225	225
Alcohol Wash, Blood	200	200
80% Ethanol	200	200
Nuclease-Free Water	50-110	50-110

Table 1. Volumes of Reagents Used in the Maxwell® HT Viral TNA method on the Tecan Freedom EVO.

6. Launch the Tecan_ViralTNA_v1_0 method in the Tecan EVOware Software.
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 Tecan Freedom EVO 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.

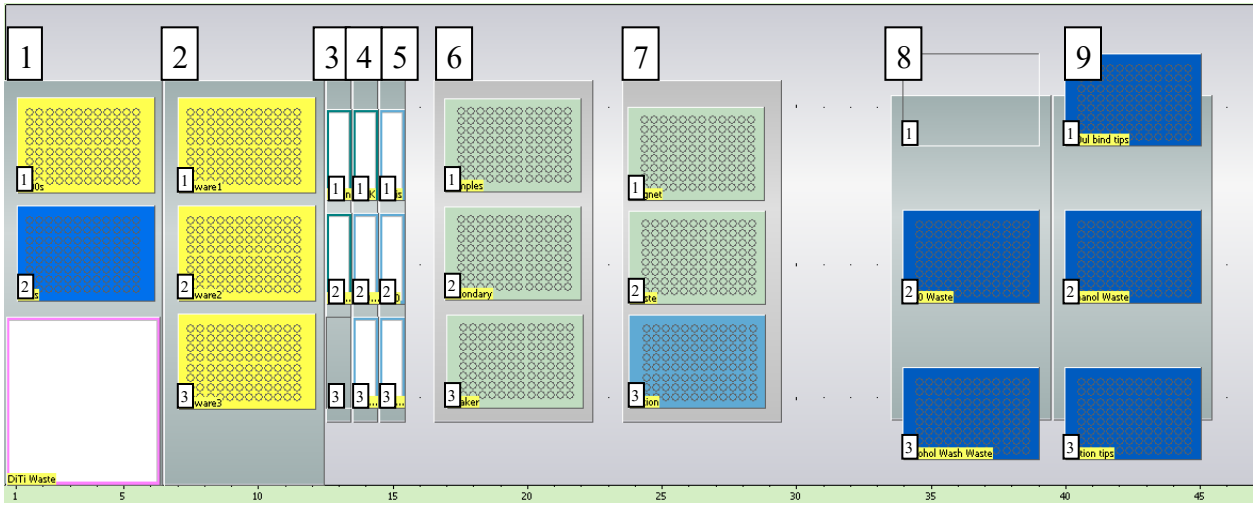

Figure 1. Deck layout for Maxwell® HT Viral TNA on the Tecan Freedom EVO 150. The deck layout is described from left to right.
Carrier 1: 1 – 1000µl Filtered tips, 2 – 200µl Filtered tips.
Carrier 2: 3 – 1000µl Filtered tip racks.
Carrier 3: 1 – 25ml Resin trough, 2 – 25ml Elution trough.
Carrier 4: 1 – 25ml Proteinase K trough, 2 – 100ml Isopropanol trough, 3 – 100ml Ethanol troughs.
Carrier 5: 1 – 100ml Lysis trough, 2 – 100ml 4/40 Wash trough, 3 – 100ml Alcohol Wash trough.
Carrier 6: 1 – Sample plate, 2 – Secondary plate (if needed), 3 – BioShake position.
Carrier 7: 1 – MagnaBot® Flex Separation Device, 2 – Waste plate, 3 – Elution plate.
Carriers 8 and 9: 200µl MCA tip boxes.

Results:

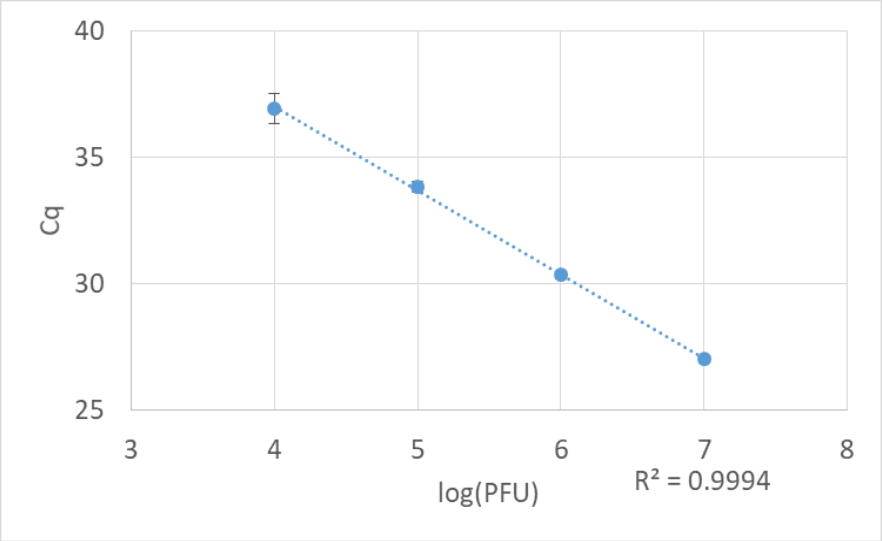

Figure 2. Linearity of nucleic acid recovery over a 4-log range of MS2 spiked into plasma samples. A set of plasma samples spiked with MS2 ranging from 10^7 PFU/200µl sample down to 10^4 PFU/200µl sample was purified in quadruplicate. Cq values were determined using the GoTaq® Probe 1-step RT-qPCR System (Cat.# A6120) and MS2 primer/probe set.

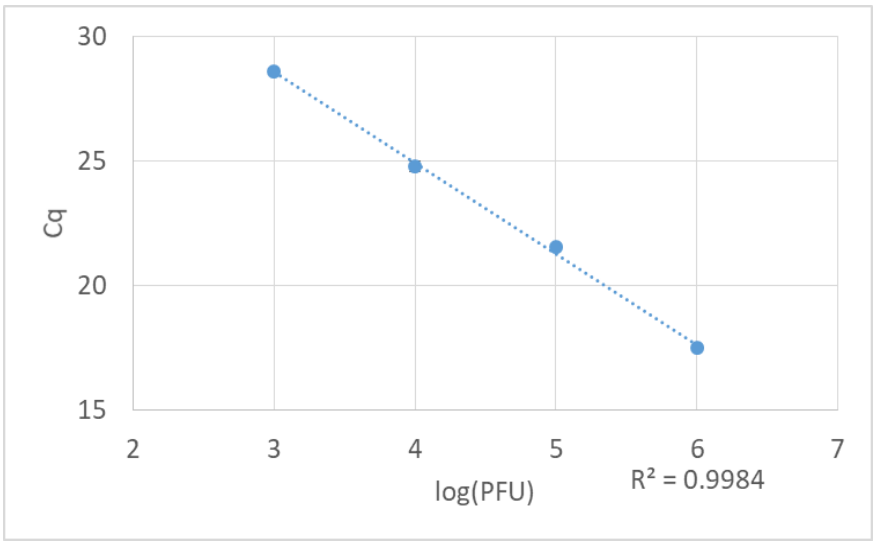

Figure 3. Linearity of nucleic acid recovery over a 4-log range of Lambda spiked into plasma samples. A set of plasma samples spiked with Lambda ranging from 10^6 PFU/200µl sample down to 10^3 PFU/200µl sample was purified in quadruplicate. Cq values were determined using the GoTaq® Probe qPCR Master Mix (Cat.# A6102) with Lambda primer/probe set.

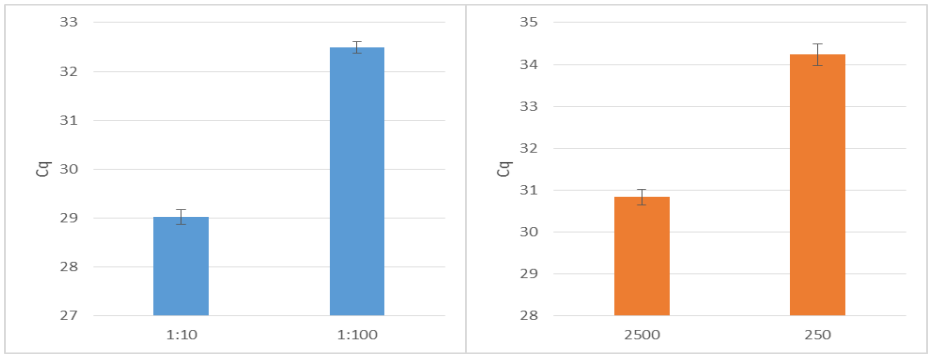

Figure 4. Nucleic acid recovery from Zika (RNA virus) and HBV (DNA virus) spiked into plasma. Zika (left) was spiked into human plasma at 1:10 and 1:100 dilutions of the provided stock. HBV (right) was spiked into human plasma at a concentration of 2500 IU/200µl sample and diluted 1:10 for a final concentration of 250 IU/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	-	31.9	-	32.0	-	32.1
B	31.8	-	31.8	-	31.9	-
C	-	32.0	-	31.8	-	32.1
D	31.9	-	31.8	-	32.0	-
E	-	32.0	-	31.9	-	32.0
F	32.0	-	31.8	-	32.0	-
G	-*	32.1	-	32.1	-	32.0
H	31.9	-*	32.0	-	31.8	-

Figure 5. Cross-contamination study. Plasma samples were spiked with MS2 at 10^6 PFU/200µl sample. 200µl of MS2-spiked or non-spiked 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 31.9 ± 0.2. Samples denoted with * showed amplification in 1 or 2 replicates above 40 Cqs. Thus, no control samples were flagged for cross contamination.

References:

1. Lanciotti, R.S., *et al*. (2008). Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007. *Emerging Infect. Dis.*, **14**:8.
2. Sun, S., *et al*. (2011). Development of a new duplex real-time polymerase chain reaction assay for Hepatitis B viral DNA detection. *Virol. J.*, **8**:227.