Promega

Product Application

High Throughput Purification of Total Nucleic Acid from Virus in plasma on the epMotion® 5075 Liquid Handling Workstation

Purify both DNA and RNA from viruses in plasma using the Maxwell® HT Viral Total Nucleic Acid Kit on the epMotion® 5075 Liquid Handling Workstation

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: 100µl 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**

Materials required:

- ep*Motion*® 5075 Liquid Handling Workstation
- TM 1000-8 eight-channel dispensing tool, 40–1000µl volume range (Eppendorf Cat.# 5280000258)
- TM 300-8 eight-channel dispensing tool, 20–300µl volume range (Eppendorf Cat.# 5280000231)
- Gripper (Eppendorf Cat.# 5282000018)
- Integrated Eppendorf Thermomixer
- Reservoir Rack (Eppendorf Cat.# 5075754002)
- Reservoir Rack 3 (Eppendorf Cat.# 5075754070)
- epT.I.P.S. Motion SafeRacks 20–300µl (Eppendorf Cat.# 0030014634)
- epT.I.P.S. Motion SafeRacks 40–1000µl (Eppendorf Cat.# 0030014650)
- Deepwell Plate 96/1000µl (Eppendorf Cat.# 951032662)
- Eppendorf™ 96-Well twin.tec™ PCR Plate (Eppendorf Cat.# 951020401)
- *epMotion*® Reservoir 30ml (Eppendorf Cat.# 0030126505)
- Reservoir 400ml (Eppendorf Cat.# 5075751364)
- Eppendorf Thermoadapter DWP 96 (Eppendorf Cat.# 5075751054)
- Deep Well MagnaBot® 96 Magnetic Separation Device (Promega Cat.# V3031)
- 100%, Isopropanol and Ethanol (molecular biology grade)

Protocol:

The procedure described below has been modified for automation on the ep*Motion*® 5075 Liquid Handling Workstation in a 96-well plate format (1ml).

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 100µl of plasma sample to the Deepwell Plate 96/1000µl.
5. The method uses the following reagents and volumes per well:

Reagents	Volume used per well (µl)
Lysis Buffer	100
Proteinase K (PK) Solution	20
Isopropanol	350
MagneSil® RED	25
4/40 Wash Solution	200
Alcohol Wash, Blood	200
80% Ethanol	200
Nuclease-Free Water	110

6. Start the run on the ep*Motion*® 5075 Liquid Handling Workstation – TNA_Viral_v1_0.
7. Set up the instrument deck as shown in Figure 1.
8. When prompted, select the number of samples to be run.
9. Add the reagents to the appropriate reagent reservoirs, as indicated in the setup screens.
10. Summary of the main steps of the *epMotion*® method:
    - Lyse sample with Lysis Buffer and Proteinase K during a heated incubation.
    - Bind nucleic acid with addition of isopropanol. Cool sample.
    - Capture nucleic acid on resin with vigorous shaking and tip mixing.
    - Remove lysate from nucleic acid-bound resin.
    - Wash resin three times with 4/40 Wash, Alcohol Wash and 80% Ethanol.
    - Dry resin thoroughly with shaking (no heat).
    - Elute nucleic acid with Nuclease-Free Water using vigorous shaking and tip-mixing.
    - Transfer eluate from the processing plate to a clean elution plate.

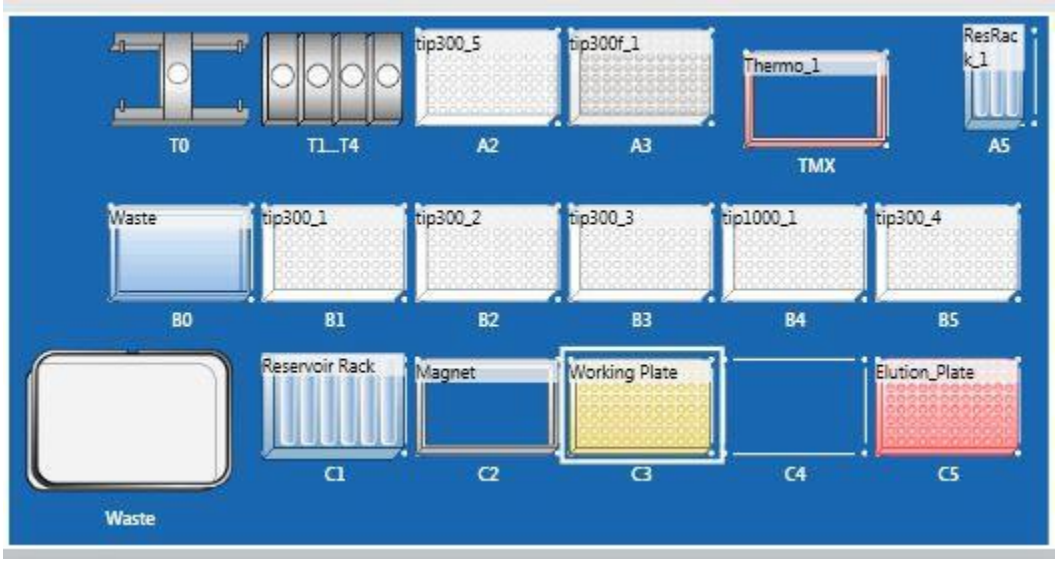

Figure 1. Deck layout for the Maxwell® HT Viral Total Nucleic Acid Kit on the ep*Motion*® 5075 Liquid Handling Workstation.

- T0 - Gripper
- T1 – T4 - TM 1000-8 eight-channel dispensing tool, 40–1000µl volume range and TM 300-8 eight-channel dispensing tool, 20–300µl volume range
- A2, A3, B1, B2, B3, B5 – epT.I.P.S. Motion SafeRacks 20–300µl
- B4 – epT.I.P.S. Motion SafeRacks 40–1000µl
- C1– Reservoir Rack (7 × *epMotion*® Reservoir 30ml)
- A5 – Reservoir Rack 3 (3 × *epMotion*® Reservoir 30ml)
- C2 – Deep Well MagnaBot® 96 Magnetic Separation Device
- C3 – Eppendorf Deepwell Plate 96/1000µl
- C5 – Eppendorf™ 96-Well twin.tec™ PCR Plate
- TMX – Integrated Eppendorf Thermomixer and Eppendorf Thermoadapter DWP 96
- B0 – Reservoir 400ml

Results:

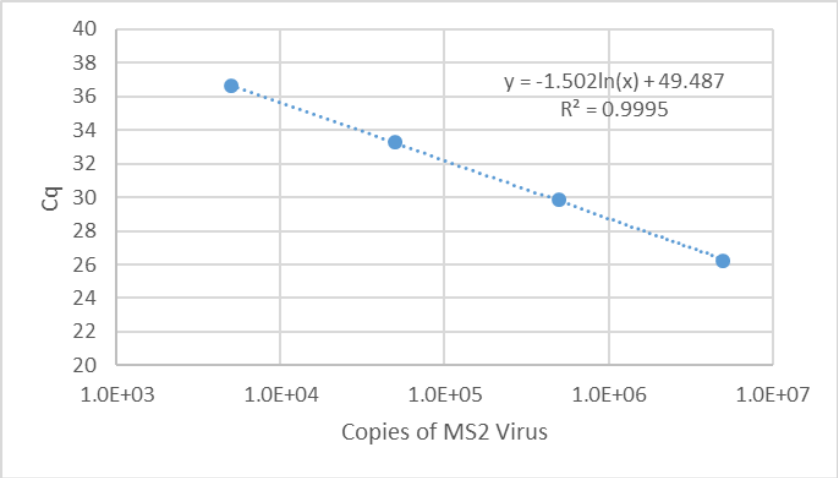

Figure 2. Linearity of nucleic acid recovery over a 4-log range of MS2 spiked into plasma samples. A set of plasma samples spiked with MS2 ranging from 5×10^6 PFU/sample down to 5×10^2 PFU/sample was purified in quadruplicate. Cq values were determined using the GoTaq® Probe 1- Step RT-qPCR System (Cat.# A6120).

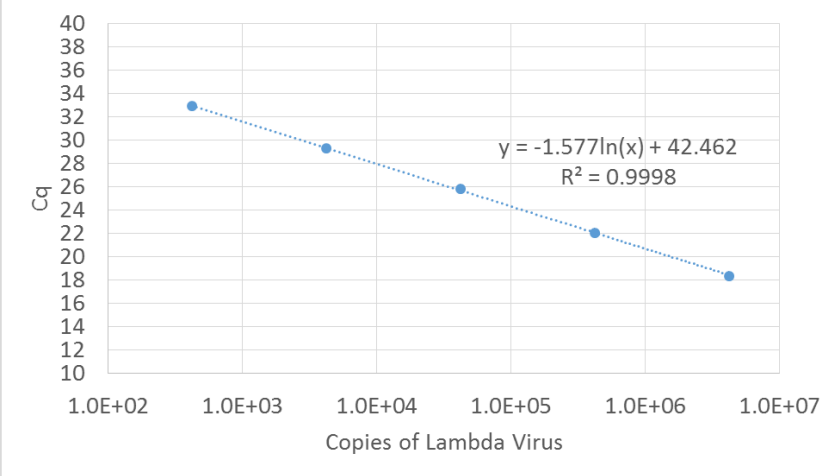

Figure 3. Linearity of nucleic acid recovery over a 5-log range of Lambda spiked into plasma samples. A set of plasma samples spiked with Lambda ranging from 4.2×10^6 PFU/sample down to 4.2×10^2 PFU/sample was purified in quadruplicate. Cq values were determined using the GoTaq® Probe qPCR Master Mix (Cat.# A6102).

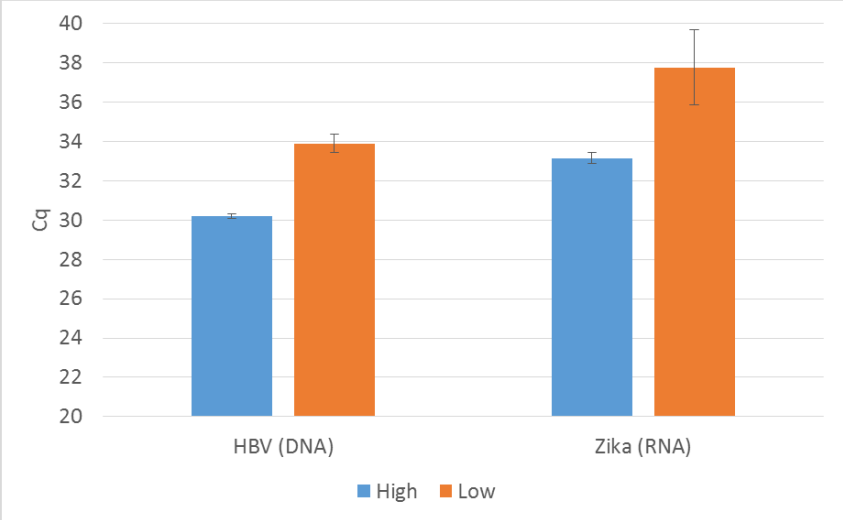

Figure 4. Nucleic acid recovery from Zika (RNA virus) and HBV (DNA virus) spiked into plasma. Zika was spiked into human plasma at 1:10 (High) and 1:100 (Low) dilutions of the provided stock. HBV was spiked into human plasma at a concentration of 1250IU/100µl sample (High) and diluted 1:10 (Low) for a final concentration of 125IU/100µl sample.

	1	2	3	4	5	6	7	8	9	10	11	12
A	31.5	-	32.9	-	33.1	-						
B	-	31.9	-	32.0	-	32.2						
C	31.4	-	31.7	-	32.1	-						
D	-	31.9	-	32.1	-	32.3						
E	31.4	-	31.7	-	32.0	-						
F	-	31.9	-	32.1	-	32.2						
G	31.5	-	31.6	-	32.3	-						
H	-	32.3	-	32.6	-	32.9						

Figure 5. Cross-contamination study. Plasma samples were spiked with MS2 at 5 × 10^5 PFU/100µl sample. 100µl 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. Following purification, detection of MS2 in each purified sample was determined using the GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6120) All amplifications were performed in triplicate. Average Cq values for each well are shown. For all wells containing MS2, each of the triplicate amplifications performed for each well was positive for MS2 with an average Cq value of 32.1 ±0.5. For all wells with plasma only, each of the triplicate amplifications performed for each well resulted in no detectable MS2 amplification out to 45 cycles. No cross-contamination was evident for the *epMotion®* method.

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.