



# Automation of the Ambion MagMAX-96 Viral RNA Isolation Kit on the Caliper Life Sciences Sciclone ALH 3000 Workstation

## Introduction

High quality viral RNA for veterinary assays is essential for rapid viral identification. For high throughput RNA isolation, solid phase binding methods using glass fiber filters and paramagnetic beads have been developed to replace phenol/chloroform extraction. The ability to perform high throughput viral RNA isolation from diverse samples is highly desirable to increase laboratory efficiency, reduce labor cost, and improve viral detection. The Ambion MagMAX magnetic bead-based technology has been successfully adapted for automated sample processing on the Caliper Life Sciences Sciclone ALH 3000 workstation.

The MagMAX-96 Viral RNA Isolation Kit can be used for viral RNA/DNA purification from a variety of animal tissues, as well as from blood, serum, plasma, milk, swabs (e.g., oral, cloacal), and feces. Small elution volumes (only 20–50  $\mu$ L) provide concentrated, purified RNA for convenient streamlining of downstream applications. The ability to perform high throughput viral RNA isolation from diverse samples enables rapid animal viral detection.

As described below, Applied Biosystems has successfully validated the MagMAX-96 Viral RNA Isolation Kit on the Caliper Life Sciences Sciclone ALH 3000 workstation. Here RNA was isolated from an Asuragen Armored RNA particle (a synthetic viral pseudo particle containing an RNA oligo) spiked into diverse biological sample types including human plasma, bovine serum, raw bovine milk, and brain heart infusion (BHI) medium (commonly used for swab sample storage). Recovery of the RNA from the Armored RNA was highly consistent across the various media types and no cross-contamination was observed.

## Materials and Methods

### Instrumentation:

Caliper Life Sciences Sciclone ALH 3000 workstation equipped with high volume head, gripper, Z-8, I/O box, and VARIOMAG® On-deck shaker

### Reagents:

- MagMAX-96 Viral RNA Isolation Kit, (# AM1836)
- 100% Ethanol
- 100% Isopropanol

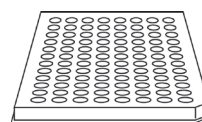
### Consumables:

- U bottom plate: provided with the MagMAX-96 Viral RNA Isolation Kit.
- 2 mL V bottom plate (Corning, #3960)
- 12 column reservoir (Innovative Microplate, #S30016)
- 150  $\mu$ L barrier 96 SBS racked pipette tips (Caliper Life Sciences, #111426)

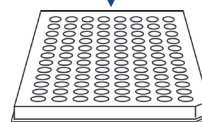
### Starting Materials:

- Lambda DNA (Applied Biosystems)
- Armored RNA® containing a Xeno™ RNA specific sequence (Asuragen)

### MagMAX-96 Viral RNA Isolation Procedure:



Biological Samples



Sample RNA

1. Add Lysis/Binding Solution
2. Add Bead Resuspension Mix
3. Shake for Five Minutes
4. Capture and Wash Beads Twice
5. Capture and Wash Beads Two More Times
6. Elute Total RNA

## Results

Figure 1A. Well Samples

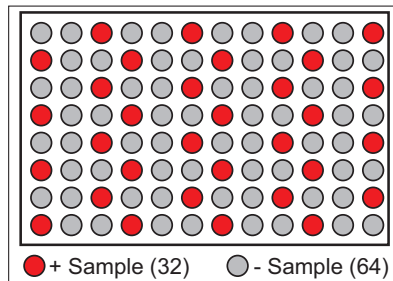


Figure 1C. Results Graph

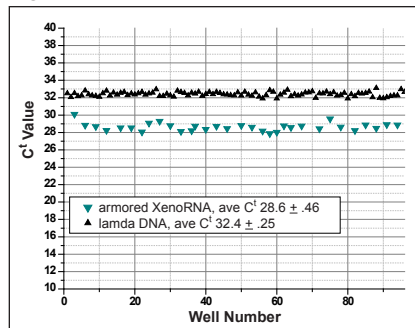


Figure 1B. Results Chart

Xeno	Human plasma			bovine serum			raw bovine milk			BHI media		
	1	2	3	4	5	6	7	8	9	10	11	12
A	***	***	30.1	***	***	28.83	***	***	28.68	***	***	28.25
B	29.09	***	***	28.54	***	***	28.54	***	***	28.06	***	***
C	***	***	29.3	***	***	28.79	***	***	28.12	***	***	28.21
D	28.73	***	***	28.36	***	***	28.71	***	***	28.46	***	***
E	***	***	28.8	***	***	28.62	***	***	28.17	***	***	28.03
F	28.77	***	***	28.57	***	***	28.75	***	***	27.87	***	***
G	***	***	29.57	***	***	28.63	***	***	28.44	***	***	28.06
H	28.87	***	***	28.49	***	***	28.92	***	***	28.22	***	***
	Average		29.16	Average		28.6	Average		28.54	Average		28.15
	Std dev		0.481	std dev		0.154	std dev		0.283	std dev		0.165

**Figure 1.** No Cross-Contamination and Highly Consistent RNA Recovery with the Ambion MagMAX™-96 Viral RNA Isolation Kit. 50 µL of human plasma, bovine serum, raw bovine milk, and brain heart infusion (BHI) medium was distributed to 3 columns of a 96-well plate. Then the Armored RNA containing a Xeno RNA specific sequence was spiked into the wells noted in red (1A) while the gray wells received no spike. The plate was then processed according to the MagMAX-96 Viral RNA Isolation Protocol using the Caliper Life Sciences Sciclone ALH 3000 workstation. Following isolation, the samples were analyzed for the presence of XenoRNA. XenoRNA target amplification results corresponded perfectly to the XenoRNA negative and positive sample well distribution (1B). \*\*\* denotes no amplification. No cross contamination was observed. To monitor consistency of recovery across the plate lambda DNA was added to the lysis/binding solution at a concentration of 0.25 ng/ sample. Analysis with qPCR targeting Lambda DNA was performed along with the qRT-PCR for the XenoRNA amplification. Amplification of lambda DNA across the plate and Amplification of XenoRNA in the spike positive wells was highly consistent with average CT of 32.4 ± 0.25 and 38.632.4 ± 0.46, respectively (1C).

## Conclusions

The Ambion MagMAX-96 Viral RNA Isolation Kit has been optimized for use with biological fluids and cell-free samples including serum, plasma, swabs, and cell culture media. RNA isolation from cell-free samples presents some unique challenges compared to RNA isolation from tissues or cultured cells. These include low viral titers, large volumes to be processed, and sample complexity. The MagMAX magnetic bead-based technology provides a fast, simple, and easily automatable method for viral RNA and DNA isolation. DNA and RNA input levels ranging from 20 copies to 25 x 10<sup>6</sup> copies can be quantitatively recovered from plasma, serum, and milk. MagMAX RNA binding beads selectively bind nucleic acids. This approach results in more consistent RNA recovery and more thorough DNA treatment than glass fiber filter-based RNA methods as the RNA binding beads can be fully resuspended in solution to enable more thorough mixing, washing, and elution. The use of Ambion MagMAX-96 Viral RNA Isolation Kit on the Caliper Life Sciences Sciclone ALH 3000 workstation provides a quick, robust method for high-throughput viral RNA/DNA isolation.

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