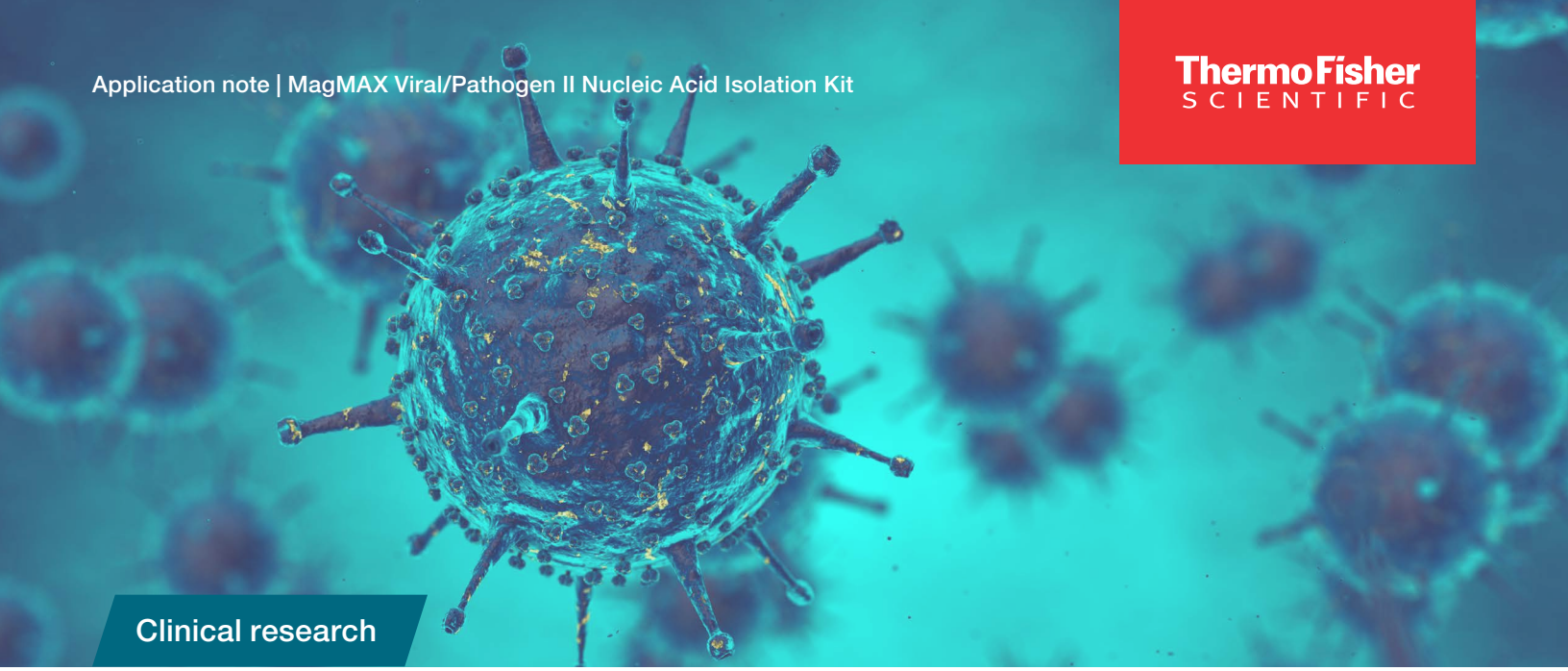

Clinical research

Inactivation of respiratory viruses with the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit

Authors

Sariah J Allen[1], Lillian Manley[1], Joy Miller[2], Carolyn Mammen[2], Shelton Bradrick[2].

[1]Thermo Fisher Scientific, Austin, TX, USA; [2]Division of Infectious Diseases, Surveillance and Diagnostics, MRIGlobal, Kansas City, MO, USA

Keywords

Viral inactivation, KingFisher instruments, nucleic acid extraction, bead-based extractions, virology, cell viability

Introduction

Viral inactivation can be beneficial in a clinical setting to improve safety and handling of specimens with infectious viral loads. There are many ways viral inactivation can be accomplished, including pH variation, chemical manipulation, heat exposure, and UV exposure. Although these methods are implemented to reduce risk of bacterial and viral contamination outside of a biocontainment hood, the methods listed add additional steps to a workflow and often require manual manipulation before starting the downstream application such as viral detection. In a clinical research setting, time and resources are often limited—it is vital to process samples accurately and quickly while minimizing exposure risk to laboratory technicians. RNA extraction with reagents containing chaotropic salts have been shown to stabilize viral RNA while preventing infectivity [1]. For example, Invitrogen™ TRIzol™ Reagent has been shown to provide successful viral inactivation by denaturing macromolecules [2]. Another approach is the use of a bead-based nucleic acid isolation kit that includes an extraction buffer that is suitable for viral inactivation. The user can transfer clinical samples in a biosafety containment hood and then immediately process the samples using semi- or fully automated techniques. This approach eliminates a separate viral inactivation step, enabling time savings while minimizing exposure of the user to the virus. The Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit effectively processes RNA and DNA viruses within various matrices for downstream applications such as qPCR. In this study, we observe the inactivation effects of MagMAX Viral/Pathogen II binding buffer on different viruses by means of $TCID_{50}$ endpoint assays and plaque assays.

Background

SARS-CoV-2 was chosen to study the effectiveness of MagMAX Viral/Pathogen II binding buffer for viral inactivation. In addition, other viruses were chosen to represent a wide variety of common human respiratory viruses found in a clinical research setting. These viruses differ in factors such as genome type, presence of envelope, virion size, and resistance to inactivation (Table 1). A common way to determine viral titer is by utilizing cell-based assays that include 50% tissue culture infectious dose ($TCID_{50}$) endpoint assays or standard plaque assays. With these techniques, serial dilution of the virus sample is required before infection of the cell monolayer. In $TCID_{50}$ assays, sample wells are scored (+/–) based on the presence (+) or absence (–) of cytopathic effect (CPE) over time to a designated endpoint. To quantify the amount of infectious virus present in the original sample, $TCID_{50}$/mL is calculated based upon the scoring. $TCID_{50}$ assays are often followed by molecular verification of viral presence by techniques such as qPCR or immunostaining. In plaque assays, plaques, or localized regions of cell death, are produced by the CPE of the virus. The cellular monolayer can be stained at this point, and viral plaques can be visually counted against the stain and quantified in plaque forming units per mL (pfu/mL) [3].

Table 1. Respiratory viruses used to test for inactivation with MagMAX Viral/Pathogen II binding buffer.

Material*	Genome type**	Average genome size (kb)	Segmented genome	Viral envelope	Average virion size (nm)	Resistance classification	Baltimore classification
2019 novel coronavirus (SARS-CoV-2), isolate hCoV-19/ Japan/TY7-503/2021	+ssRNA	29	No	Yes	Variable	Low	IV
Respiratory syncytial virus type A strain 2 (RSV-A2)	–ssRNA	15.2	Yes	Yes	150	Low	V
Human coronavirus strain 229E (HCoV-229E)	+ssRNA	32	No	Yes	115	Low	IV
Murine hepatitis virus (MHV) strain A59	+ssRNA	29.9	No	Yes	85	Low	IV
Herpes simplex virus type 1 (HSV-1) strain KOS	dsDNA	152	No	Yes	200	Low	I
Adenovirus type 5 (AdV-5) strain adenoid-75	dsDNA	36	No	No	80	Medium	I

* SARS-CoV-2 is a biosafety level-3 (BSL-3) virus. The remaining viruses are considered biosafety level-2 (BSL-2). HSV-1 was used as a surrogate for Epstein-Barr virus.

** +ssRNA: positive-sense single-stranded RNA; –ssRNA: negative-sense single-stranded RNA; dsDNA: double-stranded DNA.

Experimental procedures

Overview

BSL-2 virus inactivation was studied in-house using plaque assays. SARS-CoV-2 inactivation was evaluated in BSL-3 facilities by a $TCID_{50}$ assay for a variant strain utilizing a high-resolution cell line expressing the SARS-CoV-2 viral receptor. For BSL-2 viruses, viruses were incubated at a 1:1 ratio with MagMAX Viral/Pathogen II binding buffer at ambient temperature (15°C to 25°C) for 30 min; for SARS-CoV-2, 1:1 mixes were incubated at 10 and 30 minutes. MagMAX Viral/Pathogen II binding buffer has been demonstrated to be cytotoxic by cell-viability assays and must be removed prior to cellular infection. For BSL-2 viruses, dilution and Thermo Scientific™ Pierce™ Protein Concentrators were utilized to achieve buffer exchange, and input titer was measured via plaque assay. For SARS-CoV-2, Thermo Scientific™ Pierce™ Detergent Removal Spin Columns were used in a one-step process to minimize viral loss. In both cases, a PBS process control was used to demonstrate the buffer removal process retained infectious viral titer. Plaque assays or $TCID_{50}$ assays followed by serial passaging were used to quantify infectivity of viruses after treatment. Figure 1 shows a summary of the procedure. Table 2 contains materials utilized during these studies.

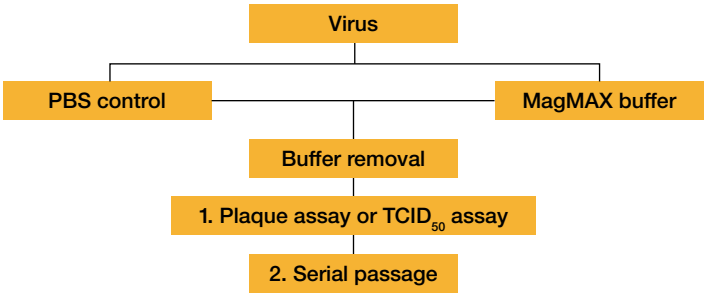

Figure 1. Viral inactivation workflows with MagMAX Viral/Pathogen II binding buffer.

Table 2. Materials used in this study.

Description	Supplier	Cat. No.
Viruses used in this study		
SARS-CoV-2, isolate hCoV-19/Japan/TY7-503/2021	BEI Resources	NR-54982
RSV-A2	BEI Resources	NR-12149
HCoV-229E	ATCC	VR-740
MHV strain A59	ATCC	VR-764
HSV-1 strain KOS	ATCC	VR-1493
AdV-5 strain adenoid-75	ATCC	VR-5
Cells and media used in this study*		
Vero E6 (DMEM, Cat. No. 11995)	ATCC	CRL-1586
Vero E6 expressing TMPRSS2 (DMEM/F-12, Cat. No. 11320)	JCRB Cell Bank	JCRB1818
HEp-2 (DMEM/F-12, Cat. No. 11320)	ATCC	CCL-23
MRC-5 (MEM, Cat. No. 11095)	ATCC	CCL-171
A549 (DMEM, Cat. No. 11995)	In-house	–
L929 (Medium 199, Cat. No. 11150)	Sigma-Aldrich	85103115-1VL

* Gibco™ medium used for maintenance of each cell line is shown in parentheses.

Plaque assays and serial passaging of BSL-2 viruses

According to literature references, plaque assays were performed utilizing either agarose or carboxymethylcellulose overlays in 12-well or 6-well plates for BSL-2 viruses [4-8]. Plaques were visualized by fixation of the monolayer with methanol or formaldehyde and staining with crystal violet. Maintenance of cell lines was conducted based on manufacturer recommendations by means of a humidified chamber at 37 ± 2°C and 5 ± 2% CO_2. Plaque assays were performed in triplicate conditions, and quantitative results were reported in mean pfu/mL. Serial passages in 12-well plates were scored (+/–) by visual monitoring for the presence or absence of viral CPE on the cellular monolayer at three time points at hours post-infection (hpi) as appropriate for each virus. For all infections, cells were observed at 0 hpi and 24 hpi to monitor for any signs of cell stress from residual buffer toxicity. Viral breakthrough below the limit of detection of plaque assays was observed by passaging cells up to two additional times.

$TCID_{50}$ assays and serial passaging of SARS-CoV-2

Inactivation of SARS-CoV-2 was evaluated by $TCID_{50}$ assays using Vero E6 cells stably expressing the SARS-CoV-2 receptor. Vero E6 cells were maintained in DMEM/F-12 containing 10% heat-inactivated FBS and antibiotics in a humidified chamber at 37 ± 2°C and 5 ± 2% CO_2. These cells were utilized for their high sensitivity in quantifying levels of SARS-CoV-2 [9]. Vero E6 cells were seeded in 96-well plates for $TCID_{50}$ assays, and 12-well plates for serial passaging on the day prior to cellular infection. Viruses underwent 10- or 30-min incubations with MagMAX Viral/Pathogen II binding buffer or PBS before buffer was removed via a one-step exchange resin to minimize viral loss. This resin also reduced the processing time needed compared to the buffer exchange process used for the BSL-2 viruses. After 1-hour adsorption of the viral inoculate, the samples were incubated in a humidified chamber at 37 ± 2°C and 5 ± 2% CO_2 for five days. Day 5 was selected as the best endpoint to confidently monitor and score viral titer for passage 1. Cells were visually scored for signs of distress at 4 hours post-inoculation to observe for the presence of CPE resulting from any buffer carryover on the cellular monolayer. $TCID_{50}$ was calculated from CPE scores using the Reed-Muench method [10]. Naïve cells grown in 12-well format were inoculated using the same conditions that were used for the $TCID_{50}$ experiments and grown for an additional 5 days to verify the presence or absence of virus below the $TCID_{50}$ assay limit of detection. This is considered to be passage 1 (P1). Upon day 5 (120 ± 4 hours), post-inoculation supernatant was used to inoculate new naïve cells in a 12-well plate format, and cells were grown for an additional 5 days—this is considered to be passage 2 (P2). A visual score for CPE was assigned to cells in the 12-well plates at 72 ± 4, 96 ± 4, and 120 ± 4 hpi. At the same time of scoring, a 50 µL aliquot was collected from each well and combined with 50 µL of DNA/RNA Shield™ Stabilization Solution, 2X (Zymo Research) for qPCR analysis of viral copies present for input (0 days) and the endpoints of each passage (5 days for P1 and 10 days for P2).

Results and discussion

Inactivation of BSL-2 viruses

Enveloped viruses such as HCoV-229E and MHV were used in a BSL-2 facility as BSL-2 surrogates for SARS-CoV-2. SARS-CoV-2 was tested in a $TCID_{50}$ assay in a BSL-3 facility as discussed in the following section. All tested respiratory viruses and viral surrogates produced intact cellular monolayers without any visible plaques after incubation with MagMAX Viral/Pathogen II binding buffer at the 10^{-1} dilution. Figure 2 shows plaque assays for the 5 viruses after inactivation in MagMAX Viral/Pathogen II binding buffer compared to the control (mixed with PBS). Serial passages confirmed the plaque assay results and demonstrated that no further viral replication occurred in cell culture after viruses were treated with MagMAX Viral/Pathogen II binding buffer. Plaque assay and serial passaging results are summarized in Table 3. Taken together, these results indicate the broad utility of MagMAX Viral/Pathogen II binding buffer in inactivating common viral respiratory pathogens.

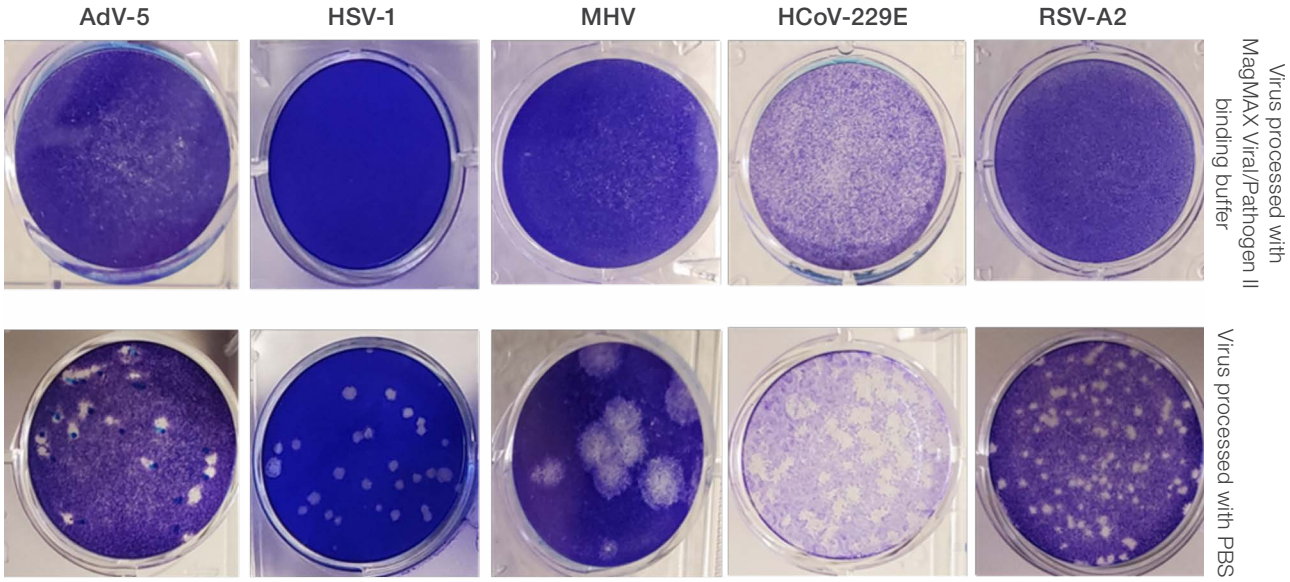

Figure 2. Plaque assay of the indicated viruses after processing in MagMAX Viral/Pathogen II binding buffer or PBS control.

Table 3. Inactivation of common respiratory viruses using MagMAX Viral/Pathogen II binding buffer. "+" indicates presence of CPE on monolayer; "<" indicates no observable titer or CPE.

	Virus input control				Buffer treatment: PBS or MagMAX Viral/Pathogen II binding buffer					
	Titer control	Passage control			Plaque assay (pfu/mL)		MagMAX Viral/ Pathogen II binding buffer, serial passages			
Virus	Plaque assay (pfu/mL)	P1	P2	P3	1:1 PBS*	1:1 MagMAX Viral/Pathogen II binding buffer	P1	P2	P3	Result
RSV-A2	1.2×10^7	+	+	+	1.7×10^5	<	<	<	<	Inactivated
HCoV-229E	2.0×10^6	+	+	+	4.8×10^4	<	<	<	<	Inactivated
MHV	9.0×10^5	+	+	+	7.7×10^3	<	<	<	<	Inactivated
HSV-1	4.0×10^7	+	+	+	1.3×10^5	<	<	<	<	Inactivated
AdV-5	1.4×10^8	+	+	+	2.0×10^6	<	<	<	<	Inactivated

* Titer is reduced after buffer treatment due to viral loss during the buffer exchange step.

Inactivation of SARS-CoV-2 variant—evaluation with Vero cells expressing viral receptor

We were unable to read the 10^{-1} dilution in all treatment conditions using MagMAX Viral/Pathogen II binding buffer due to the presence of carryover cytotoxicity in the $TCID_{50}$ plates. As such, the quantification could only begin at the dilution above that where there was still monolayer present, and the quantification may under-represent the inactivation ability of the buffer. Process controls in this experiment demonstrated high titer of SARS-CoV-2 of 7.34×10^5 $TCID_{50}$/mL (Table 4). Test conditions indicated a reduced titer of SARS-CoV-2 of $\leq 2.11 \times 10^2$ $TCID_{50}$/mL (10 min) and $\leq 1.58 \times 10^2$ $TCID_{50}$/mL (30 min). The monolayer control with buffer only (no virus did show some cell toxicity in the -1 dilution of the TCID50 assay. Serial passaging confirmed that no infectious virus could be recovered from the 10- or 30-min treatment with MagMAX Viral/Pathogen II binding buffer, suggesting that the $TCID_{50}$ value reported may be inflated due to background cytotoxicity. Additionally, an independent study performed with standard Vero cells and alpha strain of SARS-CoV-2 (isolate USA-WA1/2020) captured a reduction of 4.03 log_{10} units after only 10 min of exposure to MagMAX Viral/Pathogen II binding buffer (data not shown). It is important to note that the MagMAX Viral/Pathogen II binding buffer was tested alone, not combined with the proteinase K supplied with the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit. This is an important distinction as proteinase K inclusion would be additive to the observed inactivation effects in workflows that utilize proteinase K. The results summarized in Table 4 demonstrate the ability of MagMAX Viral/Pathogen II binding buffer to achieve high levels of viral inactivation above 4 log_{10} units, as defined by both EMA guidelines and recommendations from Public Health England [10].

Table 4. $TCID_{50}$ assay and serial passaging using SARS-CoV-2 and Vero cells expressing viral receptor incubated with PBS or MagMAX Viral/Pathogen II binding buffer. NA: not applicable; ND: not determined.

Exposure time (min)	Buffer condition	Purpose	Day 5 titer ($TCID_{50}$/mL)	Day 5 passage (viral CPE)	Day 10 passage (viral CPE)
10	MagMAX Viral/Pathogen II binding buffer + SARS-CoV-2	Test	$\leq 2.11 \times 10^2$	No	No
30	MagMAX Viral/Pathogen II binding buffer + SARS-CoV-2	Test	$\leq 1.58 \times 10^2$	No	No
30	PBS + SARS-CoV-2 (filtered)	Process control	2.11×10^5	Yes, 100%	Yes, 100%
NA	Monolayer control (no virus)	Negative control	0	No	No
NA	Virus control (unfiltered)	Positive control	7.34×10^5	ND	ND

Verification of SARS-CoV-2 inactivation by qPCR

The $TCID_{50}$ assay results were confirmed by qPCR. RNA was extracted at each time point (0 days post-infection for input, 5 days post-infection for passage 1 endpoint, and 10 days post-infection for passage 2 endpoint) and assayed for the presence of SARS-CoV-2 RNA by qPCR (standardized to viral RNA copies/mL with a standard curve). The qPCR data agree with the visual observations reported for the passaging, indicating that viral RNA copies are not observed > 5 days post-infection (dpi) with MagMAX Viral Pathogen II buffer for exposure times of 10 min and 30 min (Figure 3). Previous studies that utilized lower viral titers from the alpha variant and could only capture titers of up to 4 log_{10} $TCID_{50}$/mL also showed fully inactivated SARS-CoV-2, with no detectable copies/mL after passage, when treated with MagMAX Viral/Pathogen II binding buffer for 10 and 30 min. However, because of the current landscape of high-titer variant samples, this study was performed to increase recovered titers in the $TCID_{50}$ assay, which can be above 5 log_{10} $TCID_{50}$/mL under control conditions. These observations support a high viral-inactivation level for SARS-CoV-2 infectivity of greater than 4 log_{10} units as measured directly by viral titer in $TCID_{50}$/mL.

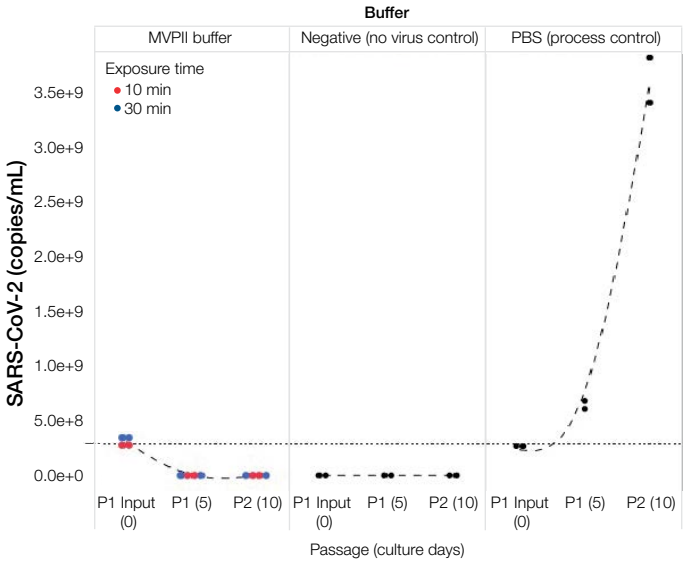

Figure 3. Confirmation of $TCID_{50}$ assays using qPCR for detection of SARS-CoV-2 variant RNA. MVPII: MagMAX Viral/Pathogen II.

Conclusions

The presented studies utilized cell-based assays to directly quantify levels of infectious virus present in treatment conditions and process controls. $TCID_{50}$ assays and serial passages confirmed that the MagMAX Viral/Pathogen II binding buffer can inactivate high levels of SARS-CoV-2 (enveloped +ssRNA virus). This result was confirmed utilizing a high-titer variant strain, a cell line expressing the viral receptor (which is fine-tuned to quantify viral replication), and infection media remaining on the cells for the duration of the assay to detect virus particles present in the treatment condition. Plaque assays and serial passages confirmed inactivation of HCoV-229E (enveloped +ssRNA human respiratory virus), MHV strain A59 (enveloped +ssRNA murine SARS-CoV-2 surrogate), RSV-A2 (enveloped –ssRNA human respiratory virus), HSV-1 strain KOS (enveloped dsDNA human virus similar to Epstein-Barr virus), and AdV-5 (non-enveloped dsDNA human respiratory virus). These results indicate that the MagMAX Viral/Pathogen II binding buffer effectively inactivates a broad range of respiratory viruses present at high levels.

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Ordering information

Product	Cat. No.
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit	A48383R
Pierce Protein Concentrator PES, 100 kDa MWCO, 5–20 mL	88532
Pierce Detergent Removal Spin Column, 4 mL	87779

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