Sera-Xtracta Virus/Pathogen Kit

DNA AND RNA EXTRACTION

Infectious diseases affect millions of people every year. Virulent and multi-drug resistant agents are increasingly responsible for infections with ever-expanding complexities. At the end of 2019, a novel coronavirus (SARS-CoV-2) was identified as the source of a pneumonia outbreak in Wuhan, China. The extraordinarily rapid spread of this disease, termed COVID-19, triggered numerous and urgent challenges to health systems, diagnostic laboratories and basic research communities throughout the world. Demand for reproducible and fast pathogen nucleic acid extraction has been unprecedented and as a result has become a limiting factor for diagnostic laboratories and research institutions globally.

The Sera-Xtracta™ Virus/Pathogen Kit* (Fig 1) provides a simple and rapid method to optimize the workflow for sensitive detection of viruses and other pathogens found in low concentrations. The silica coated magnetic bead-based automatable kit can be used with a range of sample types including respiratory biological matrices, blood and universal transport media. Using non-carrier RNA based chemistry, the kit is designed for high-throughput selective binding of total nucleic acid isolation of DNA and RNA from bacteria and viruses, including: Adenovirus (Type 14), Influenza A (H3N2) and SARS-CoV-2 (COVID-19). The isolation procedure illustrated in Figure 2 can be completed in less than 30 minutes for reproducible yields compatible with molecular biology techniques, including quantitative polymerase chain reaction (qPCR, RT-qPCR), droplet digital PCR (ddPCR), and next-generation sequencing (NGS) applications.

The following data demonstrates the proof of concept for using Sera-Xtracta Virus/Pathogen Kit for extraction of COVID-19 RNA in an experimental setting, highly relevant to virus detection from human swab samples routinely performed in a diagnostic laboratory. The experiments used synthetic viral RNA that was spiked into universal transport media containing human cells to mimic a swab sample. The detection of viral RNA in the eluant was carried out using real-time PCR (RT-PCR) assay following Centres for Disease Control and Prevention (CDC) guidelines.

Samples from suspected cases of COVID-19 are typically taken by swab and stored in universal transport media (UTM) for stabilization and transport prior to testing. A reliable and efficient RNA extraction from universal transport media

Fig 1. The Sera-Xtracta Virus/Pathogen Kit includes binding/lysis reagent, wash buffer, SeraSil-Mag™ beads and Proteinase K.

therefore constitutes a critical part of any molecular research and testing workflow. To meet this critical need Cytiva has developed Sera-Xtracta Virus/Pathogen Kit optimized for the isolation of nucleic acid from swab samples stored in PrimeStore™ MTM (FDA approved transport media, Longhorn Vaccines and Diagnostics LLC).

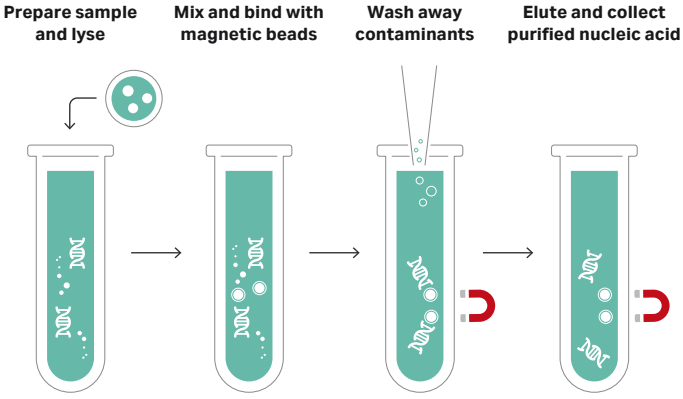

Fig 2. Nucleic acid extraction workflow with Sera-Xtracta Virus/Pathogen Kit.

*Kit is for Research Use Only (RUO)

Detection of SARS-CoV-2 RNA down to 1 copy/µL in the input sample

Synthetic viral RNA (N gene, SeraCare™) covering the nucleocapsid gene of SARS-CoV-2 was used to assess the performance and define the approximate limit of detection of the Sera-Xtracta Virus/Pathogen Kit in an experimental setting relevant to COVID-19 detection from human swab samples. Viral RNA was spiked at between 1 to 100 copies/µL into a diluent consisting of a suspension of human cells (~ 3 × 10^5/mL) in 400 µL of PrimeStore MTM to mimic a clinical sample. Un-spiked cell suspension (no viral RNA) served as the assay control to eliminate the possibility of false positives. The samples were processed using Sera-Xtracta Virus/Pathogen kit, eluted in 50 µL of nuclease-free water and subjected to Real-Time RT PCR following the CDC protocol. Briefly, extracted samples (5 µL of eluant per well) were run in technical duplicates using TaqPath™ 1-Step RT-qPCR Master Mix, CG (ThermoFisher Scientific) and CDC 2019 Novel Coronavirus (2019-nCoV) Diagnostic Panel primers (N1, N2, N3 targeting three regions of SARS-CoV-2 nucleocapsid gene and RNase P primers targeting human RNase P gene). As per CDC guidelines, only samples for which all three markers (N1, N2 and N3) crossed the threshold at Ct < 40 were considered positive; no amplification in any of the SARS-CoV-2 specific primer sets were observed in un-spiked controls (undetermined, represented as no amplification on the graph). Please note that N3 primer set has been recently removed from CDC diagnostic panel but it is still included in research use only (RUO).

The results, presented in Figure 3, confirm that the Sera-Xtracta Virus/Pathogen Kit is robust and sensitive enough for reproducible detection of low viral titer down to 1 c/µL in the input sample. For comparison with the CDC published data for the two referenced QIAGEN™ kits, please refer to Table 1 (CDC Division of Viral Diseases, CDC-006-00019).

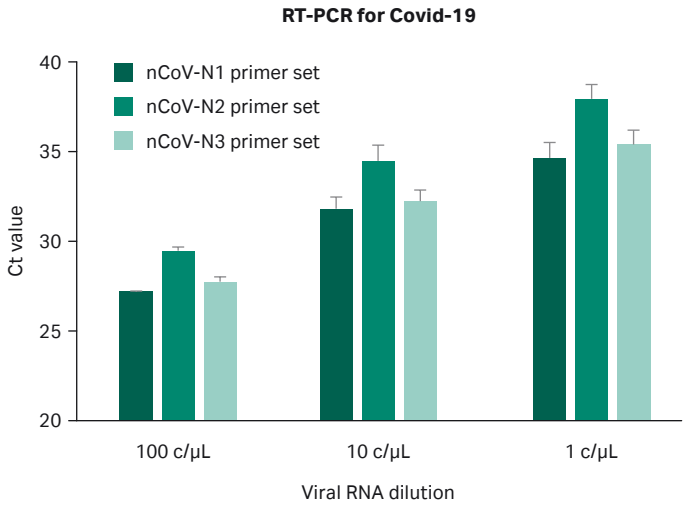

Fig 3. Ct values obtained for each of the three SARS-CoV-2 specific primer sets with varying amounts of the viral synthetic RNA in the input sample as described on the graph. Values averaged from four independent experiments with the exception of 100 copies/µL that was performed twice. Error bars represent standard deviation. Note that no SARS-CoV-2 specific amplification was detected in any of the un-spiked control samples (Ct undetermined).

Table 1. Ct values obtained for each of the three SARS-CoV-2 specific primer sets at 1 copy /µL in reference to the values published by CDC Division of Viral Diseases, CDC-006-00019 for two listed QIAGEN kits (1). Please note that the transport media identity and input volume has not been disclosed in the referenced document.

RNA concentration in input (1 c/µL)	**nCoV-N1** (Ct value)	**nCoV-N2** (Ct value)	**nCoV-N3** (Ct value)
Sera-Xtracta Virus/Pathogen Kit (Primestore MTM, 400 µL)	34.6	37.9	35.4
QIAamp DSP Viral RNA Mini Kit (VTM identity and volume not disclosed) LOD = 1 c/µL	32.8	35.4	32.7
EZ1 DSP Virus Kit (VTM identity and volume not disclosed) LOD = 3.16 c/µL	35.4	–	–

Comparative performance of Sera-Xtracta Virus/Pathogen kit

Performance of the Sera-Xtracta Virus/Pathogen Kit was compared with QIAamp™ MinElute™ Virus Spin kit (QIAGEN) using synthetic viral RNA (Microbiologics, Cat No 9009) covering the nucleocapsid gene of SARS-CoV-2. The QIAamp MinElute Virus Spin kit is based on silica columns and relies on use of carrier RNA, but was chosen as a performance benchmark based on the fact that it is recommended for the extraction of SARS-CoV-2 RNA by CDC.

Viral RNA was spiked at 1, 100, and 10^4 copies/µL into a diluent consisting of a suspension of human cells (~ 3 × 10^5/mL) in 200 µL of PrimeStore MTM to mimic a clinical sample. The samples were processed using Sera-Xtracta Virus/Pathogen Kit and QIAamp MinElute Virus Spin kit following manufacturer's protocol, eluted in 50 µL of nuclease-free water and subjected to RT-PCR following CDC protocol. Briefly, extracted samples (5 µL of eluant per well) were run in technical duplicates using TaqPath 1-Step RT-qPCR Master Mix, CG (ThermoFisher Scientific) and CDC 2019 Novel Coronavirus (2019-nCoV) Diagnostic Panel primers (N1, N2, N3 targeting three regions of SARS-CoV-2 nucleocapsid gene and RNase P primers targeting human RNase P gene).

Data from three independent experiments are presented in Figure 4 and confirm that both kits are able to detect the presence of viral RNA down to 1 copy/µL in the input sample.

Automated versus manual protocol

The Sera-Xtracta Virus/Pathogen Kit has been assessed in the KingFisher™ DUO (ThermoFisher Scientific) automation system and results compared with the manual method. Experiments were carried out using 1, 100, and 10^4 copies/µL of synthetic SARS-CoV-2 RNA in PrimeStore MTM transport media containing human cells as described previously. Extracted samples were subjected to RT-PCR following CDC protocol as described before. Data from two independent experiments from both manual and automated extraction method are shown (Fig 5) and confirm that both methods enable detection of the viral RNA down to 1 copy/µL in the input sample. The automation script is available upon request.

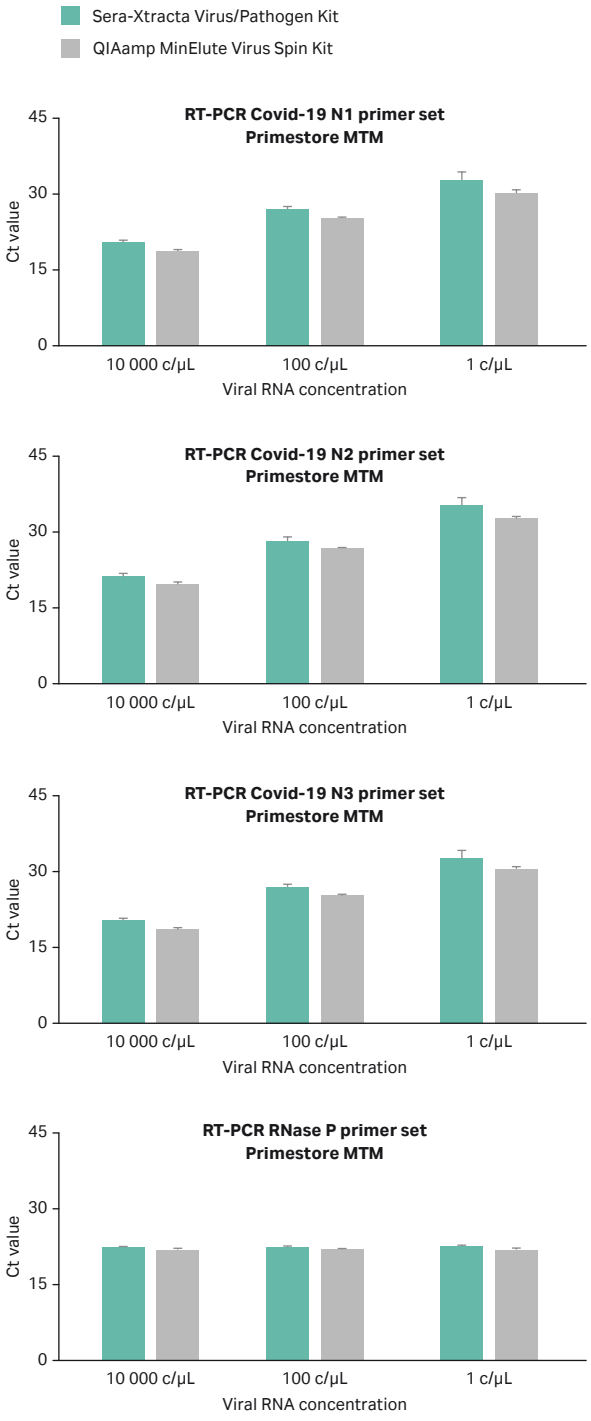

Fig 4. Ct values obtained for Sera-Xtracta Virus/Pathogen Kit and QIAamp MinElute Virus Spin kit for varying amounts of the viral synthetic RNA in the input sample as described on the graph. For clarity, Ct values obtained for each of the three SARS-CoV-2 specific primer sets (N1, N2, N3) and human-specific RNase P primer set have been presented in separate graphs. Values averaged from three independent experiments; error bars represent standard deviation.

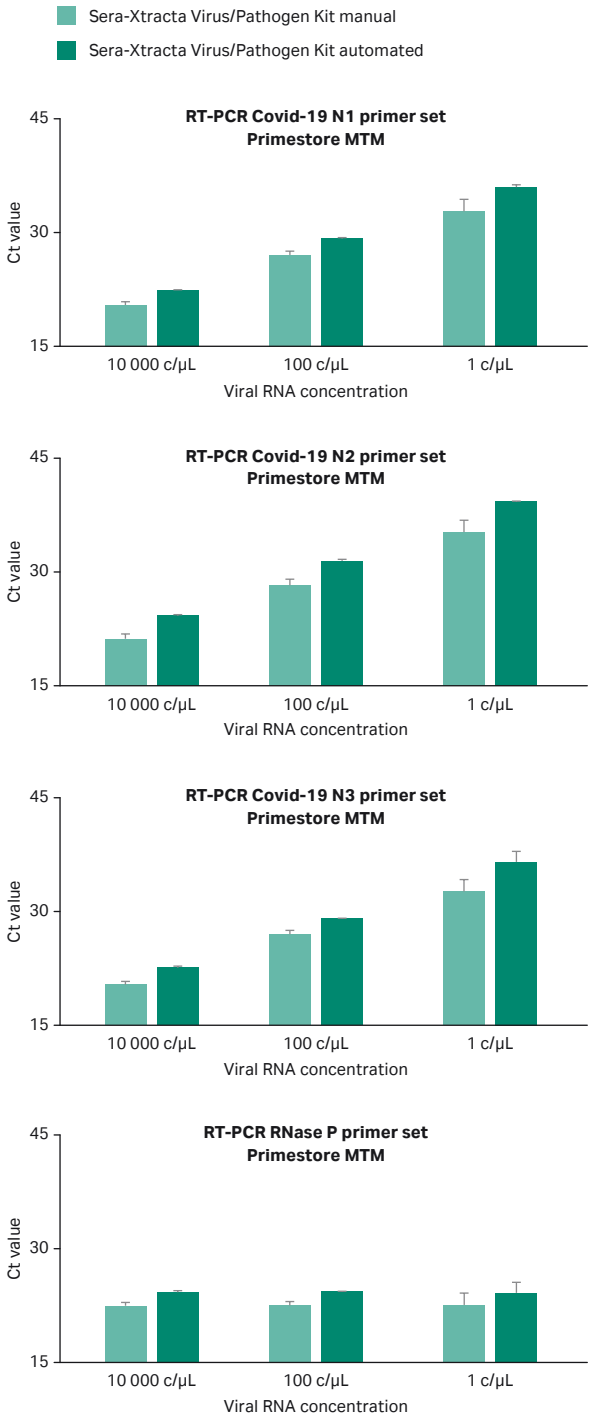

Fig 5. Ct values obtained for Sera-Xtracta Virus/Pathogen Kit in manual and automated protocol for varying amounts of the viral synthetic RNA in the input sample as described on the graph. For clarity, Ct values obtained for each of the three SARS-CoV-2 specific primer sets (N1, N2, N3) and human-specific RNase P primer set have been presented in separate graphs. Values averaged from two independent experiments; error bars represent standard deviation.

This data is based on a minimum of three independent experiments/ replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocol and recommendations. Data was collected at Cytiva, Maynard Centre, Cardiff, UK (R&D Laboratory) during March and April 2020 and is held at this location.

Performance when using other transport media

The Sera-Xtracta Virus/Pathogen Kit has been optimized for viral nucleic acid extraction from PrimeStore MTM transport media. The performance of the kit was assessed using an alternative universal transport media (UTM) in both manual and automated protocol on KingFisher DUO. 200 µL of Copan UTM (COPAN Diagnostics Inc) containing human cells (~ 3 × 10^5/mL) was spiked with a synthetic SARS-CoV-2 RNA at between 1 and 10 000 copies/µL and processed as described before. Extracted samples were subjected to RT-PCR following the CDC protocol as described previously. Data from two independent experiments are presented in Figure 6 and confirm successful detection of the viral RNA load down to 100 copies/µL in the input sample independently of the method used (automation versus manual) when using alternative transport media.

Performance using recombinant viral particles

Successful lysis and the release of nucleic acid material from viral particles are critical for the efficient extraction process and reliable detection of viral pathogens. The performance of Sera-Xtracta Virus/Pathogen Kit was tested using non-replicative recombinant Sinbis virus containing sequences from the SARS-CoV-2 AccuPlex™ (SARS-CoV-2 reference material, SeraCare) in both manual and automated protocol (Kingfisher DUO, Thermo Scientific Inc). Like Coronaviruses, Sinbis viruses contain a nucleocapsid enclosed in a lipid envelope providing a highly relevant control allowing the efficiency of RNA extraction to be extrapolated to samples containing SARS-CoV-2. The product is formulated in viral transport media mimicking a swab sample and contains approximately 5 viral copies/µL. 200 µL of SeraCare material was processed using Sera-Xtracta Virus/Pathogen kit, eluted in 50 µL of nuclease-free water and subjected to RT-PCR assay following CDC protocol described previously.

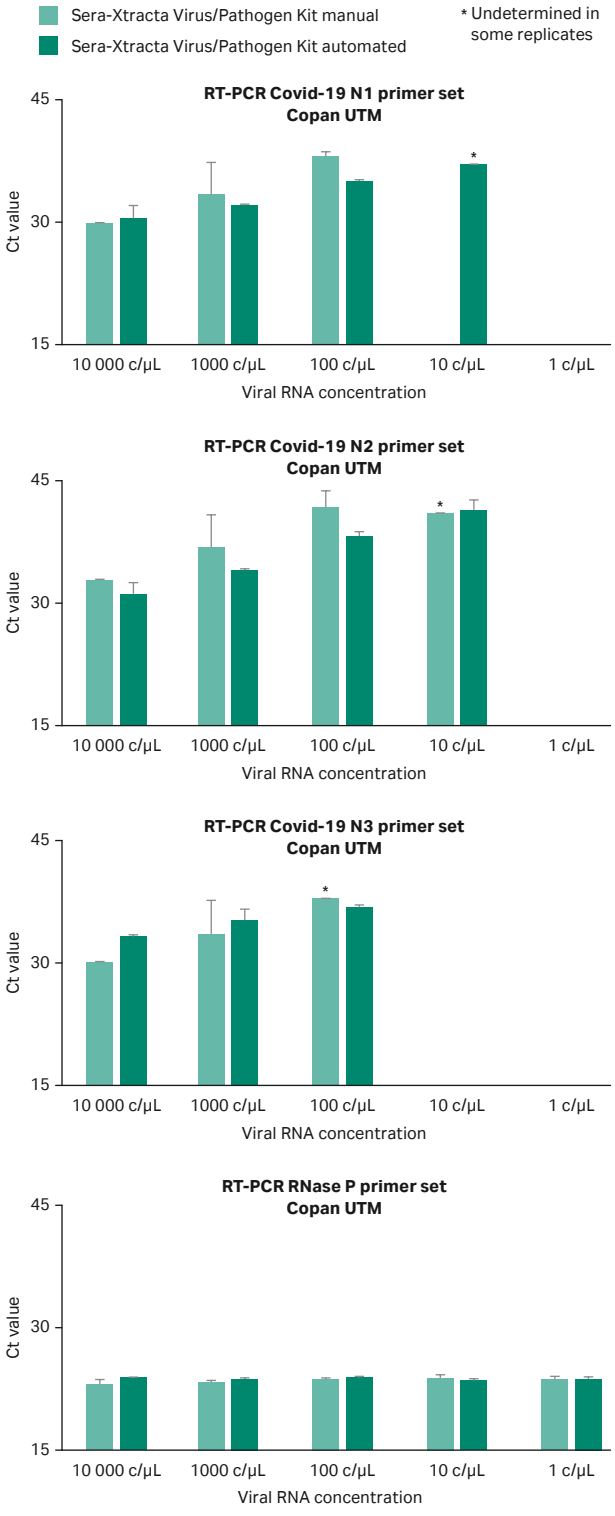

Fig 6. Ct values obtained for Sera-Xtracta Virus/Pathogen Kit in manual and automated protocol for varying amounts of the viral synthetic RNA in the Copan UTM as described on the graph. For clarity, Ct values obtained for each of the three SARS-CoV-2 specific primer sets (N1, N2, N3) and human-specific RNase P primer set have been presented in separates graphs. Values averaged from two independent experiments; error bars represent standard deviation. Please note that N3 primer set has been recently removed from CDC diagnostic panel but still included in RUO.

Data from three independent experiments are presented in Figure 7 and confirm successful extraction of RNA from viral particles, allowing for confident detection of the virus present at 5 copies/µL in the input sample independent of the method used (automated versus manual).

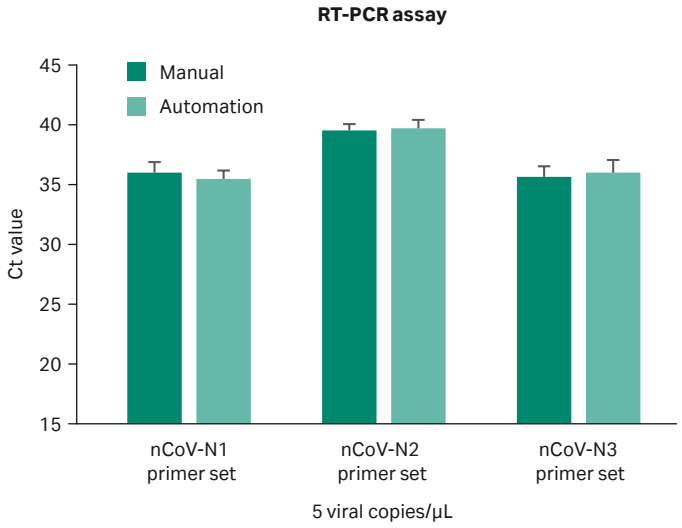

Fig 7. Ct values obtained in RT-PCR assay for 200 µL of SeraCare material containing approximately five copies of viral particles/µL processed with Sera-Xtracta Virus/Pathogen kit. Please note that no SARS-CoV-2 specific amplification was detected in the negative control (viral particles containing human RNase P sequence). For clarity, data is not shown. Values averaged from three independent experiments; error bars represent standard deviation

Conclusions

Sera-Xtracta Virus/Pathogen Kit provides an efficient, reproducible and rapid method for nucleic acid extraction of pathogens. The kit allows for detection of the viral load down to 1 copy/µL in the input sample (when using PrimeStore MTM) without the need for using carrier RNA. It is compatible with automated and manual approaches and being based on magnetic beads allows for considerable instrumentation flexibility and high throughput. The kit has been developed in response to a global COVID-19 pandemic and aimed to address current challenges faced with supplying kits for testing and scientific research. Both the format of the kit and its rapid protocol provide an excellent high-throughput solution that should aid in global efforts of the scientific and medical community to detect and study the virus.

References

1. https://www.cdc.gov/coronavirus/2019-nCoV/lab/index.html

Ordering information

Product	Pack size	Product code
Sera-Xtracta Virus/Pathogen Kit	96 purifications	29506009
Sera-Xtracta Virus/Pathogen Kit	1000 purifications	29514201

Related products

Product	Pack size	Product code
Sera-Xtracta Genomic DNA Kit	96 purifications	29429140
Sera-Xtracta Cell-Free DNA Kit	96 purifications	29437807
Sera-Mag™ Select	5 mL 60 mL 450 mL	29343045 29343052 29343057
RNASpin Mini Kit	20 preps 50 preps 250 preps	25050070 25050071 25050072
RNASpin Midi Kit	20 preps	25050073
SeraSil-Mag 400	5 mL 60 mL 450 mL	29357369 29357371 29357372
SeraSil-Mag 700	5 mL 60 mL 450 mL	29357373 29357374 29357375
Poly(A)	100 mg 500 mg	27411001 27411002
MagRack 6	1.5 mL/2.0 mL microtubes	28948964

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