

A Rapid, Simple, and Low-Cost RNA Sample Preparation Method Based on Chelex™ 100 Resin

Elizabeth Dreskin,¹ Angelica Olcott,¹ and Ertan Ozyamak¹

¹Bio-Rad Laboratories, Inc., 6000 Alfred Nobel Drive, Hercules, CA 94547, USA

Abstract

Chelex 100 Resin is a heat-stable bead polymer that captures divalent cations, such as Mg²⁺ and Ca²⁺, from solutions. This property allows the inhibition of nucleases in biological samples, thus preserving DNA or RNA molecules for downstream detection. Here, rapid protocols using Chelex 100 Resin and a related product, InstaGene Matrix, were compared against a commercial RNA extraction kit for sample preparation from a variety of sample types for use in reverse transcription quantitative PCR (RT-qPCR) testing.

Introduction

Frequent ongoing surveillance is an effective strategy to minimize or prevent community spread of infectious diseases. However, several factors such as high cost and insufficient throughput can hamper such public health efforts. To aid in community surveillance studies, a low-cost, rapid, and simple protocol for RNA sample preparation based on Chelex 100 Resin, a chelating resin, was devised. Treatment with Chelex 100 Resin captures divalent cations, inhibiting nucleases in biological samples.

Chelex 100 Resin is a bulk resin, which can be prepared at the concentration or percentage of the user's choice, and is a product often used for higher throughput needs. For standard laboratory

applications, Chelex 100 Resin is available as InstaGene Matrix, a small volume (20 ml), ready-made 6% Chelex 100 Resin suspension. Either Chelex 100 Resin or InstaGene Matrix can be used to prepare RNA samples simply by mixing the product with the sample and performing a short heat treatment (Figure 1). The supernatant can then be used directly for PCR, RT-qPCR, reverse transcription Droplet Digital™ PCR (RT-ddPCR), or reverse transcription loop-mediated isothermal amplification (RT-LAMP).

Our data show that a range of sample types prepared with Chelex 100 Resin or InstaGene Matrix yield amplifiable RNA and genomic DNA comparable to that obtained using a commercial extraction kit at a fraction of the cost and preparation time.

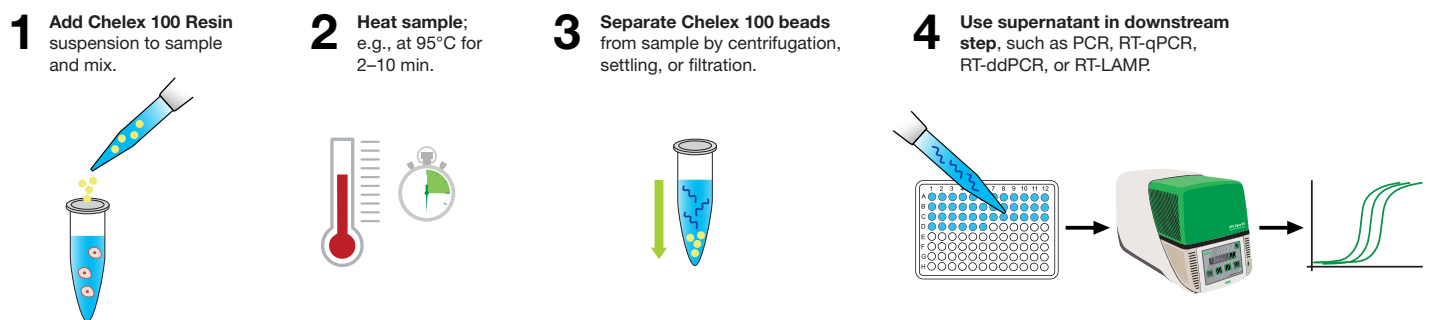


Fig. 1. Chelex 100 Resin-based sample preparation workflow overview. RT-qPCR, reverse transcription quantitative PCR; RT-ddPCR, reverse transcription Droplet Digital PCR; RT-LAMP, reverse transcription loop-mediated isothermal amplification.

Materials and Methods

Samples of A549 cells (adenocarcinomic human alveolar basal epithelial cells), cheek buccal cells, and saliva were prepared in Virus Transport Media (VTM) (Neuromics, catalog #VTM-4) that was spiked with the synthetic RNA template at 1/10 of the final volume. Cheek buccal cells were isolated from the inside cheek area by scraping with a sterile pipet tip. Saliva samples were obtained by direct transfer into a standard laboratory tube. For viral RNA detection tests, influenza virus A was used at 2x, 4x, and 6x the previously determined limit of detection (LOD).

Chelex 100 Resin, molecular biology grade (Bio-Rad Laboratories, Inc. #1421253), was prepared at a 6% w/v suspension in Tris-EDTA (TE) buffer (pH 8.0) or double distilled water (ddH₂O), followed by three washes of the same before being brought to final volume in TE buffer or water. Chelex 100 Resin in ddH₂O was used after initial testing because TE buffer showed similar results. InstaGene Matrix, 20 ml (Bio-Rad, #7326030) is a ready-made qualified 6% suspension of Chelex 100 Resin. Fifty microliters of either suspension was added to each 50 µl sample. The bead suspensions were vortexed after every three dispenses to keep the resin beads from settling prior to pipetting. For convenience, 8-well PCR strip tubes were used. Once combined, the Chelex 100 Resin and InstaGene Matrix samples were placed in a thermal cycler and incubated at 95°C for 10 min. Samples were cooled to 4°C, centrifuged briefly for several seconds in a microcentrifuge to sediment the beads, and then 50 µl of supernatant was transferred into a fresh tube, making sure to avoid disturbing the sedimented beads. To compare results obtained using Chelex 100 Resin and InstaGene Matrix to a commercial kit, the QIAamp Viral RNA Mini Kit (QIAGEN, #52904) was used according to the manufacturer's instructions with 50 µl of sample per replicate. Nucleic acids were eluted off the column such that the final volume for each replicate was 50 µl.

RT-qPCR was carried out using Reliance One-Step Multiplex RT-qPCR Supermix and several different assays run on a Bio-Rad real-time thermal cycler. To detect genomic DNA (gDNA), the RNase P Assay (Cy5.5; Bio-Rad, #12004601) was used. The PrimePCR Reverse Transcription Control Probe Assay (FAM; Bio-Rad, assay ID: qHsaCtIP0001001; referred to hereafter as the PrimePCR RT Control Assay) was used to detect spike-in RNA, and the PrimePCR Probe Assay: ACTB (HEX; Bio-Rad, assay ID: qHsaCEP0036280) was used to detect endogenous RNA. Influenza A was detected using a proprietary probe assay designed against the M1 gene. Reactions (20 µl each) were set up using 10 µl of master mix and 10 µl of template. Spike-in experiments used the synthetic RNA template that ships with the PrimePCR RT Control Assay. The following protocol was used for RT-qPCR thermal cycling: 50°C for 10 min, 95°C for 10 min, followed by 40 cycles of 95°C for 10 sec and 60°C for 30 sec. For all experiments, three replicate preparations were made, and each preparation was analyzed by RT-qPCR in triplicate.

For Chelex 100 Resin and InstaGene Matrix, the final sample volume was 100 µl, whereas samples extracted using the QIAamp Kit were eluted to a final volume of 50 µl. Input sample volume was kept consistent for all methods (see previous paragraph). Quantification cycle (C_q) values were adjusted to reflect the differences in sample volume.*

For RNA stability tests, sample aliquots were stored at 4°C after preparation with InstaGene Matrix or a QIAamp Kit as described above. Sub-aliquots were removed to a plate with RT-qPCR master mix at 1, 2, 3, and 4 hr timepoints, then evaluated by RT-qPCR to determine RNA integrity over time.

*To account for the higher dilution of the sample, the obtained C_q values for Chelex 100 Resin and InstaGene Matrix were manually decreased by 0.85 cycles. This value was determined by estimating the difference in final sample volume when using the resin-based methods versus the kit-based method. We confirmed the use of C_q adjustment across several samples and it was equivalent to bringing the recovered sample volumes to the same level.

Results

RNA Preparation from Cultured Cells

Our data demonstrate that combining cell suspensions with Chelex 100 Resin or InstaGene Matrix and heating at 95°C lyses cells and yields amplifiable RNA, genomic DNA, and spike-in RNA (when used). This shows that Chelex 100 Resin and InstaGene Matrix work effectively to chelate out divalent cations that may promote nucleic acid degradation or interfere with reverse transcription or DNA polymerase activities.

Data from 500 cell and 5,000 cell inputs are shown in Figure 2. From these results one can conclude that Chelex 100 Resin and InstaGene Matrix perform similarly to each other and that the preparation method is suitable for downstream analysis of both RNA and DNA. Additionally, both products perform comparably to the QIAGEN QIAamp Kit.

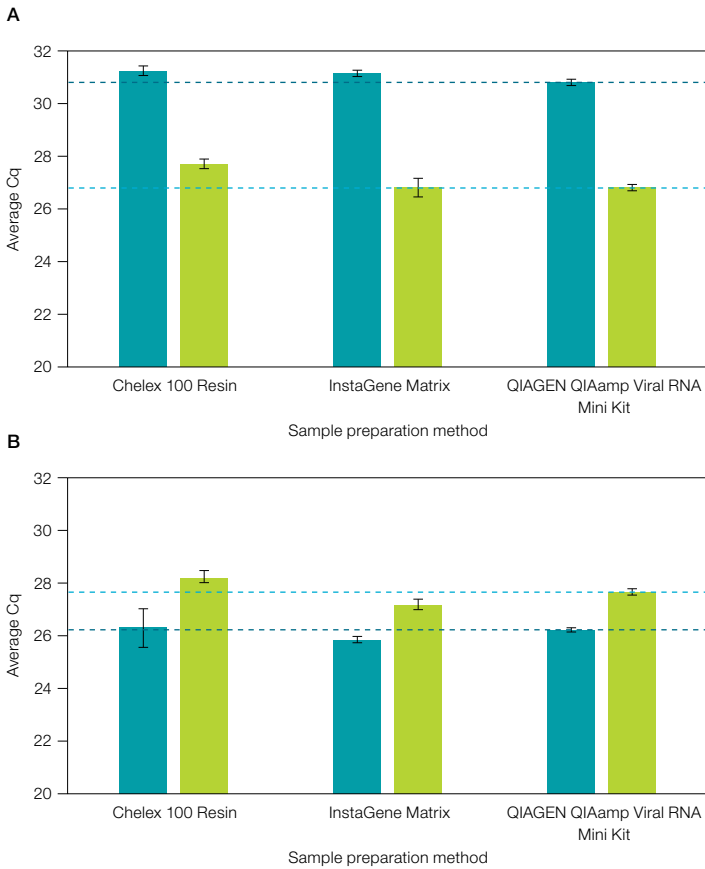


Fig. 2. Comparison of Chelex 100 Resin, InstaGene Matrix, and QIAGEN QIAamp Viral RNA Mini Kit for extraction of genomic DNA and spike-in RNA. Comparison of average Cq values for genomic DNA (■) and the spike-in RNA control (■) for samples prepared with Chelex 100 Resin, InstaGene Matrix, or a QIAGEN QIAamp Kit. Each starting sample consisted of: **A**, 500 A549 cells; **B**, 5,000 A549 cells. Results are averages of three samples per treatment, each assayed by reverse transcription quantitative PCR (RT-qPCR) in technical triplicate. Error bars indicate standard deviation. The average Cq values for spike-in RNA (—) and genomic DNA (—) prepared using the QIAamp Kit are marked for reference. Cq, quantification cycle.

Chelex 100 Resin and InstaGene Matrix perform almost as well as the QIAamp Kit for spike-in reverse transcription (RT) control RNA, genomic DNA, and endogenous RNA over a wide range of cell concentrations (from 5 to 50,000 cells per 50 µl) as shown in Figure 3. Each data point shows the averaged results of three samples per treatment, each assayed in three technical replicates by RT-qPCR, yielding a total of nine measurements for each sample.

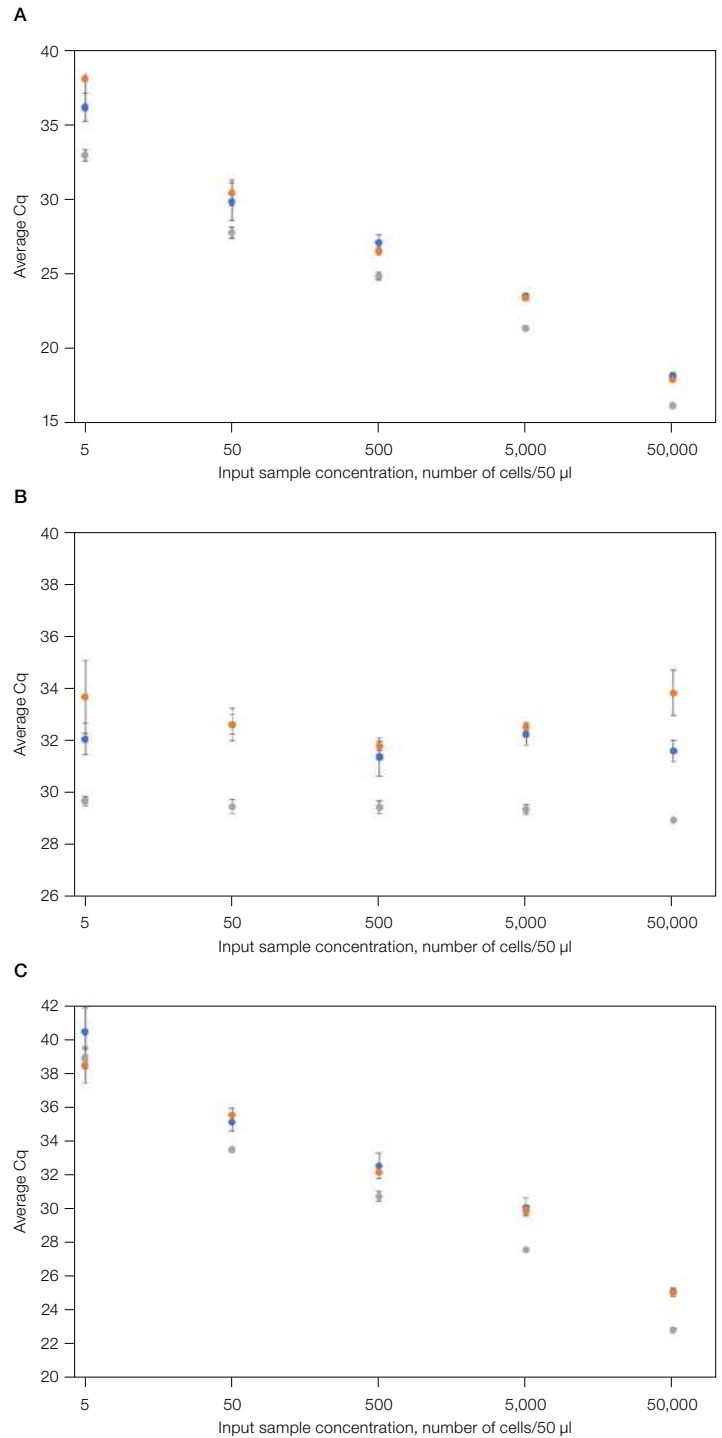


Fig. 3. Comparison of nucleic acid extraction methods at varying cell input ranges. Chelex 100 Resin and InstaGene Matrix work well for sample preparation over a wide range of input A549 cell numbers. Graphs show average Cq values obtained for endogenous RNA (**A**), spike-in RNA (**B**), and genomic DNA (**C**) samples after preparation with InstaGene Matrix (●), Chelex 100 Resin (●), or a QIAGEN QIAamp Viral RNA Mini Kit (●). Error bars indicate standard deviation. Cq, quantification cycle.

Stability of Prepared Samples

The stability of the prepared samples at 4°C for up to 4 hours was tested to determine whether this method could potentially work for sample preparation in assays that have specific sample type requirements. The data presented in Figure 4 indicate that samples prepared with InstaGene Matrix over a range of input cell counts are stable. InstaGene Matrix and Chelex 100 Resin were shown to be equivalent in terms of the stability of extracted samples at 4°C (data not shown).

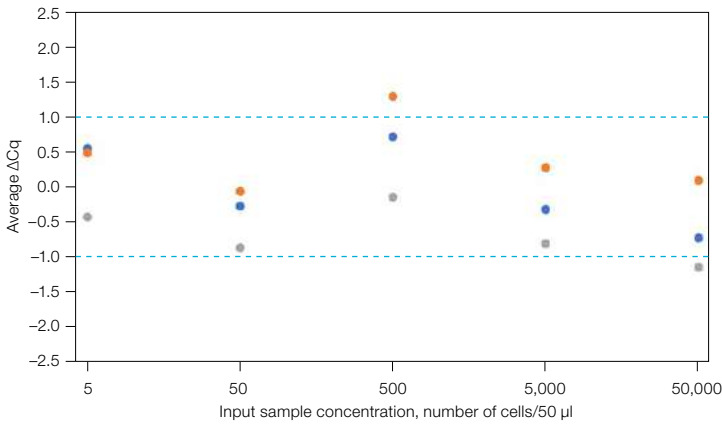


Fig. 4. RNA stability of samples using InstaGene Matrix. Samples prepared with InstaGene Matrix are stable at 4°C for at least 4 hours postpreparation. The average change in quantification cycle (ΔCq) values between prepared samples added to RT-qPCR master mix immediately and samples added after being stored at 4°C for 4 hr is plotted on the y axis against the number of input A549 cells. Average ΔCq values are shown for endogenous RNA (●), genomic DNA (●), and spike-in RNA (●), and a boundary of $\pm 1.0 \Delta Cq$ is marked for reference (—).

Preparation of Other Sample Types

The Chelex 100 Resin-based method was evaluated for sample preparation of RNA and DNA from influenza A virus (Figure 5, Table 1), as well as cheek scrapes and saliva (data not shown). For influenza A samples, template was detectable by RT-qPCR in Chelex 100 Resin-treated samples even when input quantities as low as twice the LOD were used, indicating that this is a suitable method for quick and efficient preparation of virus samples.

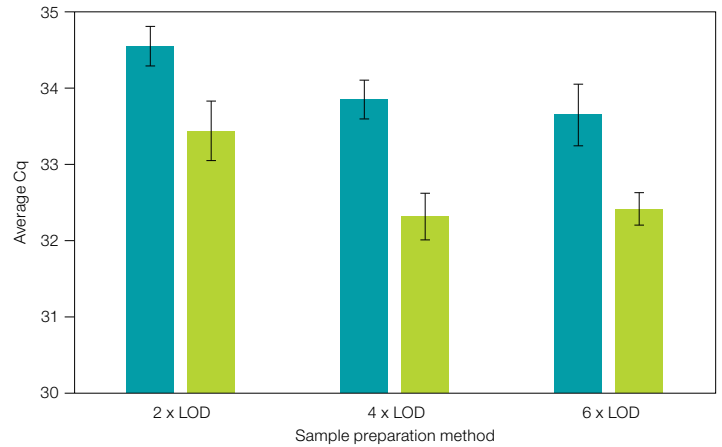


Fig. 5. InstaGene Matrix is effective at sample preparation for viral RNA detection. Average Cq values obtained from reverse transcription quantitative PCR (RT-qPCR) analysis of samples containing influenza A viral RNA. Samples were prepared using InstaGene Matrix (■) or a QIAGEN QIAamp Viral RNA Mini Kit (■). Cq, quantification cycle; LOD, limit of detection.

Table 1. Comparison of Cq values obtained for detection of influenza A in prepared viral RNA samples using InstaGene Matrix versus a QIAGEN QIAamp Viral RNA Mini Kit.

Viral RNA Input Quantity	QIAGEN QIAamp Kit		InstaGene Matrix		
	Average Cq	SD	Average Cq	SD	Average ΔCq
2 x LOD	34.6	0.261	33.4	0.387	1.1
4 x LOD	33.9	0.252	32.3	0.307	1.5
6 x LOD	33.7	0.406	32.4	0.211	1.2

LOD, limit of detection; Cq, quantification cycle; SD, standard deviation; ΔCq , difference in Cq values.

For epithelial cells isolated from cheek buccal scrapes and saliva samples, treatment with InstaGene Matrix could enable detection of both spike-in RT control and genomic DNA (data not shown). Owing to the presence of known PCR inhibitors and RNAses, RNA levels are expected to be lower in saliva samples, and the resulting Cq levels higher, compared to other sample types such as epithelial cells from cheek buccal scrapes (Ostheim et al. 2020).

Workflow Time, Cost, and Plastic Usage

A major benefit of the Chelex 100 Resin-based methods is their cost effectiveness. Researchers reported an estimated eightfold cost reduction for SARS-CoV-2 detection when using Chelex 100 Resin-based methods, including the cost of collection tubes and additional reagents (Guan et al. 2021). Another study indicated that the cost of Chelex 100 Resin required per sample was 50- to 170-fold cheaper than the per sample cost for traditional DNA extraction kits or reagents (Lienhard and Schäffer 2021).

Factoring in only the reagent cost, we estimate savings in the range of 18- to 36-fold per sample when InstaGene Matrix is used as described in this protocol compared with RNA extraction kits from major manufacturers, as of the time of this publication. Further significant savings can be achieved with Chelex 100 Resin.

In addition to the reduced cost, the workflow enabled by Chelex 100 Resin or InstaGene Matrix also offers substantial reductions in both the time required for sample preparation and the generation of biohazardous and plastic waste (Figure 6).







	Sample preparation time	Cost	Waste generation	Application
RNA extraction kits				RT-qPCR ✓ RT-ddPCR ✓ RT-LAMP ✓
Chelex 100 Resin				RT-qPCR ✓ RT-ddPCR ✓ RT-LAMP ✓

Fig. 6. Comparison of sample preparation time, cost, plastic waste generation, and application suitability for Chelex 100 Resin-based methods versus a commercial-grade kit for RNA or DNA isolation. RT-qPCR, reverse transcription quantitative PCR; RT-ddPCR, reverse transcription Droplet Digital PCR; RT-LAMP, reverse transcription loop-mediated isothermal amplification.

As a result, the use of Chelex 100 Resin or InstaGene Matrix can streamline a given molecular detection workflow and significantly reduce costs with the added benefits of excellent sustainability due to far lower waste generation. Chelex 100 Resin-based sample preparation methods are suitable for RT-qPCR (shown here; Ulloa et al. 2020), RT-ddPCR (Guan et al. 2021), and RT-LAMP (Yaren et al. 2021).

Conclusions

Commercial DNA and RNA extraction kits isolate nucleic acids at high enough purity and yield for use in molecular assays and are considered the gold standard for detection by RT-qPCR. However, certain applications may not justify the high cost and slow processing speed (low throughput) associated with such kits. Here we demonstrated that both Chelex 100 Resin and InstaGene Matrix, which stabilize RNA/DNA in samples through chelation of divalent cations, provided results highly comparable to a commercial RNA extraction kit from QIAGEN. InstaGene Matrix and Chelex 100 Resin offer several substantial benefits for RNA

sample preparation from mammalian cells and viruses: the cost per preparation is significantly lower than available RNA extraction kits, the protocol is rapid, and much less plastic waste is generated per preparation. In cases where a larger number of samples will be run consistently, Chelex 100 Resin may be a preferred option due to bulk pricing advantages.

The additional advantage of Chelex 100 Resin is the ability to customize the suspension buffer as well as the suspension concentration. In this study, we chose a 6% (w/v) Chelex 100 Resin suspension, however, suspensions of 10–20% are typically used in sample preparation protocols and suspensions of up to 50% have been reported. A higher Chelex 100 Resin concentration may yield improved results in cases where a higher capacity to capture divalent ions, and therefore to limit nuclease activity, is needed. Overall, for community surveillance purposes, Chelex 100 Resin and InstaGene Matrix offer compelling advantages over other commercial kits, especially when availability of supplies is limited.

It should be pointed out that because the Chelex 100 Resin–based protocols do not isolate nucleic acids, the reagents used in RT-qPCR may play a critical role in the detection of the RNA or DNA targets queried. We have found that Reliance One-Step Multiplex RT-qPCR Supermix can dependably detect these targets when the sample accounts for 50% of the final reaction volume. Because this reagent is supplied as a 4x solution, it is theoretically possible to add sample volumes larger than 50% of the final reaction volume; however, this condition was not tested here.

This method is potentially automatable using a liquid handler with an on-deck heating block or a thermal cycler that can handle 96-well and 384-well plates as well as microfuge tubes. Therefore, this method may also enable higher throughput screening.

References

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- Lienhard A and Schäffer S (2019). Extracting the invisible: Obtaining high quality DNA is a challenging task in small arthropods. *PeerJ* 7, e6753.
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- Ulloa S et al. (2020). A simple method for SARS-CoV-2 detection by rRT-PCR without the use of a commercial RNA extraction kit. *J Virol Methods* 285, 113960.
- Yaren O et al. (2021). Ultra-rapid detection of SARS-CoV-2 in public workspace environments. *PLoS One* 16, e0240524.

Ordering Information

Catalog #	Description
1421253	Chelex 100 Chelating Resin , molecular biology grade, 200–400 mesh, sodium form, 50 g
7326030	InstaGene Matrix , 20 ml

Visit [bio-rad.com/Chelex100-RNA](https://www.bio-rad.com/Chelex100-RNA) for more information and a list of publications using Chelex 100 Resin.

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