GALENVS

June 2020

magnetiQ

Viral RNA Extraction Kit Handbook

For in vitro diagnostic use for COVID-19 and R&D applications

Galenvs Sciences Inc.
6750 Hutchison Street, Suite 209
Montreal, QC, Canada H3N1Y4
Phone: (438) 368-4160
Email: contact@galenvs.com
Website: www.galenvs.com

IVD CE

Galenvs Magnetic Technologies

Galenvs Sciences Inc. is a Canadian biotechnology company based in Montreal, Quebec, Canada offering turn-key platforms and solutions for researchers and clinicians in academic, government, biotech, and pharmaceutical labs. Galenvs specializes in magnetic-based nucleic acid isolation formulations generated by machine learning technology to obtain superior products.

Galenvs provides magnetic reagent and kits with superior efficiency for:

- Viral RNA Extraction
- Total RNA Extraction
- Blood and Cell DNA Extraction
- PCR Clean-Up
- Plasmid Miniprep

Galenvs' mission is to supply the life sciences R&D community with tools that address complex biomedical challenges through simple and elegant solutions.

For more information, visit **www.galenvs.com**

Contents

Intended Use

The intended use of the *magnetiQ* Viral Extraction Kit is for research purposes or diagnostics (IVDD) of COVID-19 in clinical laboratories for the extraction of RNA from patient samples for later quantification via Reverse Transcriptase qPCR (RT-qPCR).

The kit is intended for use in a laboratory by a trained laboratory professional proficient in molecular biology diagnostics.

Samples can include VTM, ITM, serum/plasma, saliva or cell-free bodily fluids.

For complex matrices such as blood or sputum, please consult the FAQ on page 19.

This kit does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay.

Summary and Explanation

The Galenvs *magnetiQ* Viral Extraction Kit allows for one-step magnetic bead-based extraction and purification of Viral RNA under 15 minutes from:

- Swab solutions (viral transport medium - VTM, and inactivation transport medium - ITM)
- Serum/plasma
- Saliva samples
- Cell-free body fluids
- For complex matrices such as blood or sputum, please consult the FAQ on page 19

Each kit contains (i) lysis/binding, (ii) wash #1, (iii) wash #2, and (iv) elution buffers — (v) as well as RNA carrier — to obtain purified concentrations of viral RNA free of debris, salt, lipid and protein contamination. The kit is available in 2 formats allowing for processing of 100 (VR1010) and 250 extraction/reactions (VR0250).

The Galenvs *magnetiQ* Viral Extraction Kit does not require the use of benchtop equipment. Only a magnetic rack and RNAase-free microfuge tubes (not included; sold separately) are needed to conduct the RNA isolation protocol. Ethanol (not included) must be added to the wash #1 and #2 buffers prior to use. It is important that all buffers - especially the lysis/binding buffer - are mixed thoroughly by inverting up and down until homogeneous before commencing each extraction protocol.

This kit can be stored at room temperature for over 12 months post manufacture date, with the exception of the RNA carrier component that must be stored at 4°C- 8°C for short-term storage (up to 3 months) and -20°C for long-term storage (up to 1 year).

Principles of the Procedure

The *magnetiQ* Viral RNA Extraction Kit is based on magnetic bead technology for the capture and purification of viral RNA. All Galenvs reagents and buffers are also optimized using machine learning approaches and are the only AI-powered kits on the market. The RNA purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples. The extraction procedure comprises 3 steps: lysis/binding, washing, and elution (see page 7).

Lysis/binding

The lysis/binding buffer incorporating magnetic beads is added to the specimen sample to achieve the digestion of the viral coat proteins and the inactivation of nucleases while simultaneously providing the optimal conditions for capture of the viral nucleic acids on the magnetic beads.

Washing

Following lysis/binding of the viral RNA to the magnetic beads, a series of washes is then performed to remove any contaminating proteins, lipids and salts. This is performed through magnetic capture of the beads using a magnetic rack and the subsequent removal of the supernatant, followed by resuspension in the appropriate buffer according to the outlined protocol.

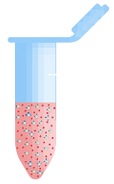
Elution

A final elution step is then performed to obtain purified RNA ready for downstream analysis using any molecular analysis techniques such as RT-qPCR for the gene targets of interest.

- In addition to manual operation, the *magnetiQ* Viral Extraction Kit is also amenable to automation using any commercially available platform that allows operation with third party reagents, for magnetic liquid handling such as the Kingfisher platform from Thermofisher.

Galenvs *magnetiQ* Viral RNA Extraction Kit

1



Sample collection: swab solutions, serum/plasma, saliva samples, cell-free body fluids

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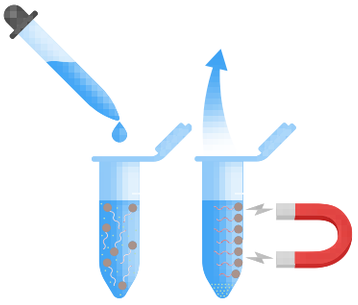

Lysis and binding of RNA material to magnetic beads

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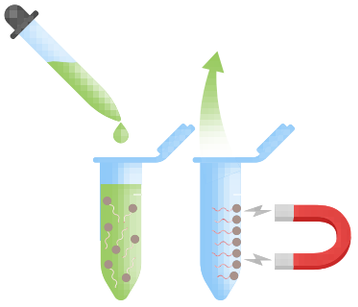

Washing of contaminating proteins, lipids, and salts from magnetic beads

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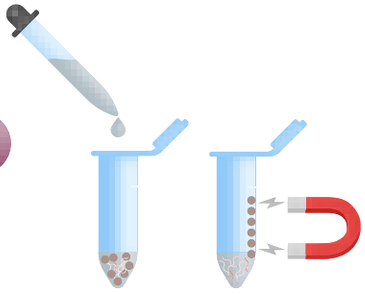

Elution of RNA Magnetic Beads

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Highly purified viral RNA

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Downstream analysis using molecular biology techniques

Kit Contents

Materials provided

	Quantity /Volume for 100 prep kit (Cat. VR1010)	Quantity /Volume for 250 prep kit (Cat. VR2050)
Viral Lysis/Binding Buffer*	40 mL	100 mL
Wash Buffer #1	21 mL	52.5 mL
Wash Buffer #2	42 mL	105 mL
Elution Buffer	15 mL	20 mL
Carrier RNA	250 µL	625 µL
Quick Start Guide	1	1
Handbook	1	1

**Contains a guanidine salt that can cause skin irritation. Not compatible with disinfectants containing bleach. See page 9 for safety information.*

Materials required but not provided

Please use suitable protection equipment when working with chemical reagents. When working with the *magnetiQ* Viral RNA Extraction Kit it is recommended to always wear a lab coat, disposable gloves, and protective goggles.

- Ethanol (>95% purity)
- Magnetic racks
- Micropipettes
- Standard Sterile, RNase-free pipette tips
- Standard microfuge tubes

Warnings and Precautions

This kit is suitable only for research purposes and for *In Vitro* Diagnostic of SARS-CoV-2.

Always wear a suitable lab coat, disposable gloves, and protective goggles when working with chemicals. For more information, please consult the appropriate safety data sheets (MSDSs). These are available online in PDF format at **galenvs.com/product/viral-rna-extraction**

CAUTION: the viral lysis/binding buffer contains hazardous material (guanidine salts) and should be handled with extreme caution.

CAUTION: NEVER add bleach or acidic solutions directly to preparation waste containing guanidine salts.

Some buffers contained in the kit contain guanidine salts, which can form highly reactive compounds when combined with bleach. In case of spills, clean liquid with detergent and water.

Galenvs has not tested the liquid preparation waste generated after the Virus RNA extraction procedure for residual infectious materials. Contamination of the preparation liquid waste with residual infectious materials is highly unlikely due to inactivation of the virus with the lysis/binding buffer during the first step of the extraction procedure, however this cannot be excluded completely. Therefore, any residual liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

CAUTION: Samples may be potentially infectious and must be handled with care by trained laboratory professionals. Any generated waste from viral RNA extraction protocol must be discarded in the appropriate proper biohazard waste.

The following hazard and precautionary statements apply to the components of this Viral extraction kit:

Contains: alcohol and guanidine salts; Danger! Causes severe skin burns and eye damage. Highly flammable liquid and vapor. Dispose of contents/container to an approved waste disposal plant. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF ON SKIN (or hair): Remove/take off immediately all contaminated clothing. Rinse skin with water/ shower. Immediately call a POISON CENTER or doctor/ physician. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Store in a well-ventilated place. Keep cool. Wear protective gloves/protective clothing/eye protection/face protection.

Reagent Storage and Handling

All buffers can be stored at room temperature (15-25°C). The magnetic particles in the lysis/binding buffer remain active when stored at this temperature. Do not freeze the buffers. When stored properly, all kit reagents are viable up to 1 year post manufacture date.

Only the RNA carrier is required to be stored at 4°C for short-term storage (up to 3 months) or at -20°C for long-term storage (up to 1 year). If storing RNA carrier for long-term use, it is recommended to aliquot the solution and store at -20°C in order to avoid repeated freeze/thaw cycles.

Precipitates may form in the lysis/binding buffer due to the presence of magnetic particles after storage even at proper store conditions. It is important to resuspend the particles before each extraction procedure by vigorously shaking the bottle for a few seconds.

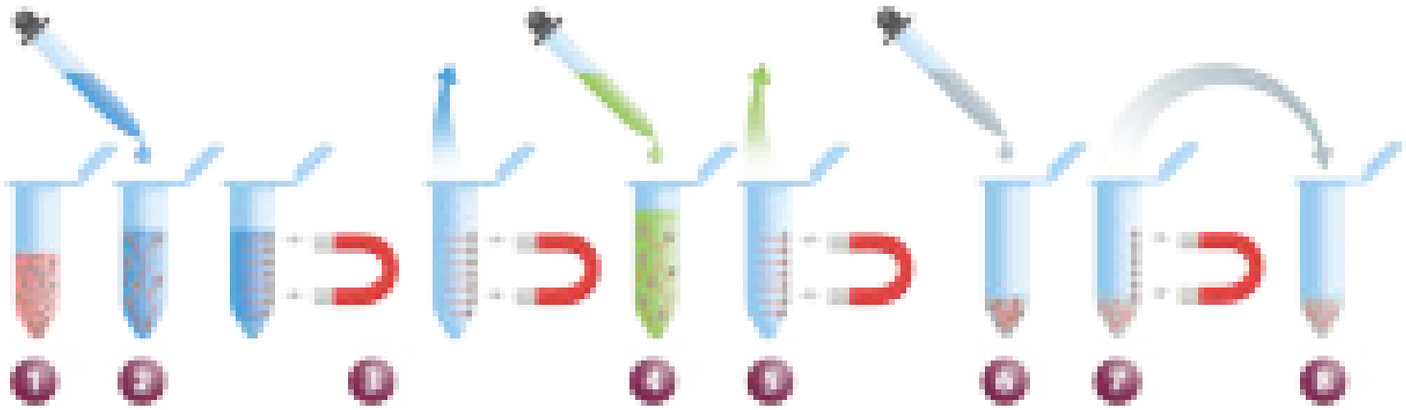
Protocol

The following protocol is for viral nucleic acid extraction from viral transport medium - VTM, and inactivation transport medium - ITM), serum/plasma, swab solutions, or any cell-free body fluids.

The protocol can be applied at the indicated volumes for any of the listed sample matrices in order to obtain a highly purified viral RNA solution for downstream analysis.

No pre-treatment of the listed sample types is necessary prior to extraction protocol.

It is important to note that ethanol (>95%) must be added to wash #1 and #2 as per label instructions prior to first use of the kit. A check mark box is provided on wash #1 and #2 bottle top labels to serve as reminders that ethanol (>95%) has indeed been added to respective bottles.



* Before first use, add ethanol (>95%) to wash buffers #1 and #2 as per label instructions
* Prior to extraction experiment, mix bottles well by inverting upside down several times

1. In 1.5 mL of microfuge tube, add 100 µL of sample (serum/plasma, swab solutions, cell-free body fluids, viral transportation media (VTM), inactivation transport media (ITM), urine, or culture supernatants)
   Optional: if extracting/purifying low RNA concentrations, add 2.5 µL of **Carrier RNA** to sample
2. Add 400 µL of **Viral Lysis/Binding Buffer** and mix well by pipetting up-down 10-15x.
   Incubate 5 mins to allow for lysis and RNA binding
3. Place tube on magnetic rack for 1-2 mins to capture RNA-bead complex, then discard supernatant
4. Remove tube from magnetic rack and resuspend RNA/bead complex in 600 µL of **Wash Buffer #1**
5. Return to magnetic rack for 5 mins, then discard supernatant. Repeat wash with 600 µL **Wash Buffer #2**, return to magnetic rack for 1-2 mins, then discard supernatant and leave to dry for 1 min
6. Remove tube from magnetic rack and resuspend RNA-bead complex in 50 µL of **Elution Buffer**.
   Mix well by pipetting up-down 15-20x to elute RNA from beads and let stand for 1-2 mins
7. Place tube on magnetic rack to separate beads (~1-2 mins)
8. Transfer clean RNA solution (supernatant) to clean tube

Notes:

Use of the RNA carrier

The RNA Carrier serves two purposes during the purification procedure. First, it enhances binding of viral nucleic acids to the magnetic particles, especially if the sample contains very few copies of target RNA. Second, the addition of the RNA carrier reduces the chances of target viral RNA degradation in the unlikely event that RNases are not completely denatured by the chaotropic salts and detergent in the lysis/binding buffer. If the RNA carrier is not added to the reaction, recovery of viral RNA may be significantly reduced.

Elution volumes

The final step of the purification procedure is the elution of viral RNA in a final volume of 50 µl. However, this volume could be adjusted depending on the specific application or downstream analysis. It is important to keep in mind that diluting the final concentration of the extracted RNA by increasing the elution volume will cause a shift in obtained values during downstream analysis.

Storage of purified viral nucleic acids

For short-term storage of up to 24 hours, we recommend storing the purified viral RNA at 2–8°C. For long-term storage of more than 24 hours, we recommend storage at –80°C to –20°C.

Performance characteristics

To evaluate the extraction of the *magnetiQ* Viral RNA Extraction Kit for isolation and recovery of SARS-CoV-2 RNA, validation was performed by the National Research Council of Canada (CNRC-NRC) –Medical Devices (MD-DM). Detailed results are presented in the Appendix A of this document.

Quality Control

In accordance with Galenvs Quality Assurance, each lot of viral RNA extraction kit is tested against predetermined specifications to ensure consistent product quality.

Limitations

It is the user's responsibility to validate the kit performance for any procedures used in their laboratory that are not covered by the Galenvs performance evaluation studies.

In case of clinical use, the kit performance has been established in performance evaluation studies using **viral transport medium - VTM, inactivation transport medium - ITM), serum/ plasma, saliva, or any cell-free body fluids** for extraction and purification of SARS-CoV-2 viral RNA. To minimize the risk of a negative impact on the diagnostic results, adequate controls to avoid contamination for downstream applications should be used. It is recommended that any diagnostic results generated must be interpreted in conjunction with other clinical or laboratory findings.

References

For any references or technical assistance, visit the Galenvs website at **www.galenvs.com** or contact your local distributor.

Contact Information

Galenvs, with its in-house scientific team, is deeply committed to the end-user customer experience.

For any product questions or feedback: **www.galenvs.com/contact**

Troubleshooting Guide and Frequently Asked Questions

Before using the *magnetiQ* Viral RNA Extraction Kit, it is important to add ethanol (>95%) to wash buffers 1 & 2 as per label instructions. It is recommended to mark the bottle top label to indicate ethanol has been added.

Before each extraction experiment, make sure that all bottles are well mixed by inverting upside down several times.

Caution! Magnetic racks contain a very powerful magnet that could cause physical harm if not handled carefully. Also, avoid placing the magnet near electronic equipment such as computers and cellphones.

1) How many microfuge tubes should be used for each extraction?

The whole extraction protocol should be performed in a single tube. It is recommended to transfer the clean RNA eluate to a new tube for storage or downstream experiments.

2) How many extractions should be performed using magnetic racks?

Each extraction in a single tube can be performed in 15-20 minutes and needs a single magnetic rack. If high throughput is required, many extractions can be performed simultaneously using several magnetic racks in parallel. However, it is recommended to perform 8 extractions at a time with 8 racks as increasing the number of extractions can lead to unwanted user error.

3) Can any commercial magnetic rack be used?

Yes, any commercially available magnetic rack will be compatible. However, depending on the magnet strength, capture time may vary.

4) What sample types can be used for viral RNA extraction?

Any cell-free media can be used for viral RNA extraction. This includes swab preservation solution, viral inactivation media, viral transport media, cell culture supernatants (ie. DMEM or other media), and saliva.

5) When should carrier RNA be used?

It is recommended to use carrier RNA if the viral load is very low. Galenvs recommends using carrier RNA if the viral copy number is below 100 copies/mL of sample.

6) Can water be used for elution?

Nuclease-free water or DEPC-treated water may be used for elution if required.

7) Can elution volume be more or less than 50µL?

50uL is the recommended volume as it allows for an appropriate concentration of RNA for downstream processing (e.g. PCR, NGS, etc). However, less volume (20-50µL) can be used to obtain a more concentrated sample. Also, more volume (up to 200µL) can be used to obtain more dilute eluate if needed.

8) Is heating or vortexing necessary for viral RNA extraction?

No heating or vortexing is required for the *magnetiQ* Viral RNA Extraction Kit protocol.

9) Can vortexing be used for mixing instead of pipetting up/down?

It is recommended to mix by pipetting since vortexing may cause the sample to be trapped under the lid of the microfuge tube. However, vortexing can be used if prefered but doing so, in turn, will require a short spin/centrifugation to retrieve and collect all the samples from the microfuge tube's lid. This centrifugation step should be performed for a very short period of time (few seconds) to avoid magnetic particle aggregation.

10) Which pipette tip sizes should be used?

For carrier RNA addition, 1-10µL tips should be used; For sample, 10-100µL or 200uL tips should be used; For lysis/binding and wash buffers, 100-1000µL tips should be used; For elution, 10-100 or 200µL tips should be used.

11) After discarding supernatant following wash #2 and before elution, there is still liquid remaining. Is 1 minute enough to dry beads before elution?

It is important to try to discard all remaining solution following wash #2. Usually, 1 min drying should be enough but can be increased to 3 minutes if necessary. Do not leave to dry for over 5 minutes as the beads become too dry – some wetness is still necessary for efficient elution.

12) Why should the capture for Wash #1 be for 5 minutes and only 1-2 minutes following Wash #2?

We recommend incubation on the magnetic rack following wash #1 of 5 minutes to ensure that any contaminants will be desorbed from the bead surface. This is not necessary for wash #2, as the beads have become cleaner and the only removal of salts is required.

13) Can the kit be used for sample matrices containing cells, such as sputum, mucus, or blood?

Yes, the kit may be used as well for cell-containing media such as sputum, mucus, blood or any media containing high protein amounts. However, the addition of proteinase K (20mg/mL) solution to the sample - at 20uL per 100uL sample - is strongly recommended. This greatly helps in nuclease digestion and denaturation of nucleic acid-binding proteins. In short, it is recommended that the sample (100uL) be supplemented with 20uL of proteinase K, as well as 2.5uL of RNA carrier, prior to the addition of 400uL viral lysis/binding buffer.

Appendix A: Validation of *magnetiQ* Viral RNA Extraction Kit for COVID-19 RNA Isolation

Purpose

To evaluate the extraction of *magnetiQ* Viral RNA Extraction Kit for isolation and recovery of SARS-CoV-2 RNA. Validation was performed by the National Research Council of Canada (CNRC-NRC) – Medical Devices (MD-DM).

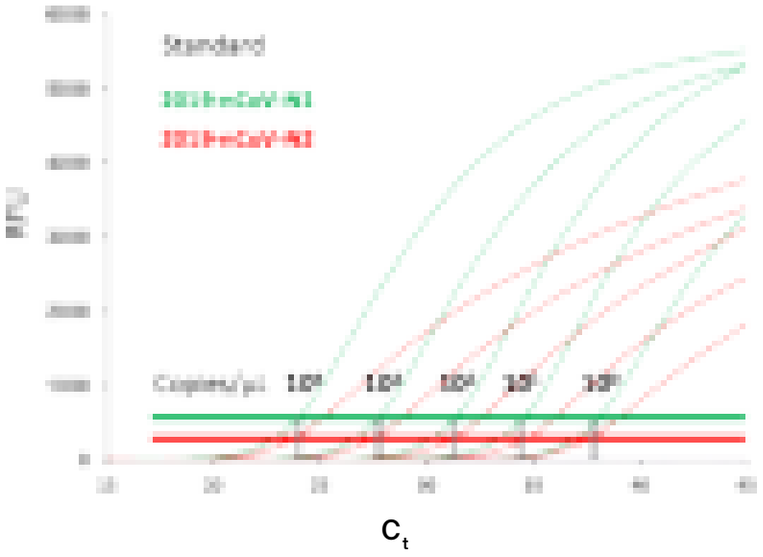
Method

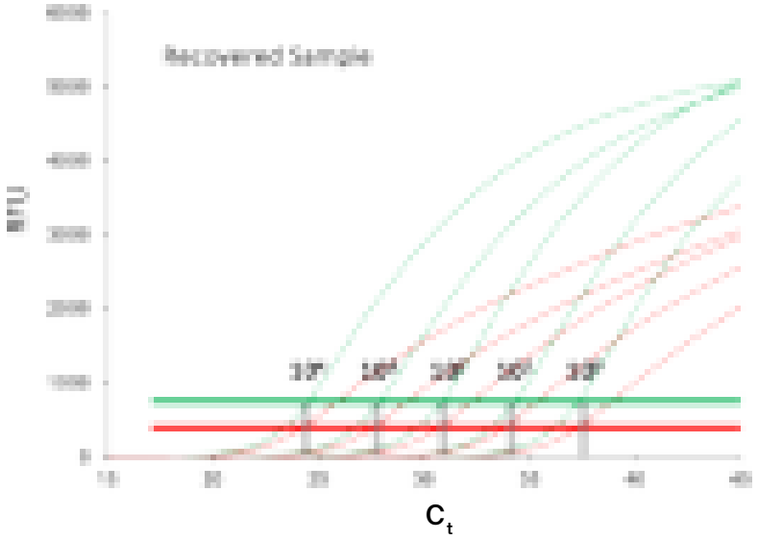
Current sample collection is performed using swab that is inserted in a standard Transport Inactivation Media (ITM). Synthetic SARS-CoV-2 RNA (Twist Bioscience) was spiked in standard preservation solution at varying dilutions between 104 and 1 copy/µL. Sample solutions were prepared at a volume of 100 µL, and the Galenvs protocol was followed for RNA capture and elution at 50 µL. The capture and extraction efficiency was then evaluated using RT-qPCR and compared against a standard curve for calibration purposes. A dual-plex assay – based on the CDC 2019-nCoV panel – was employed wherein primers and hydrolysis probes for N1 and N2 genes, supplied by Integrated DNA Technologies (IDT), are included for detection in FAM and HEX, respectively.

Results

It is critical for RNA extraction to be efficient at low copy concentrations in order to achieve early viral detection. In addition, the eluted samples should possess high purity to allow for routine RT-qPCR analysis. A commercial RT-qPCR mix from ThermoFisher (TaqPath 1-Step Master Mix) was used according to manufacturer protocol. Thermocycling and analysis was performed using a Biorad CFX96 instrument and CFX Maestro software.

The Galenvs kit was evaluated according to the provided protocol. Following sample elution and RT-qPCR analysis, the performance of the Galenvs kit showed high extraction efficiencies of 99-100% recovery for low SARS-CoV-2 copy numbers ranging from 1-100 copies/µL. This is of significant importance for detection of viral loads commonly associated with early onset COVID-19 infections, which are reported to be between 1-10 copies/µL, as outlined by Health Canada, FDA, CDC and WHO.





	Threshold Cycle C_t		
Concentration	Standard	Recovered Sample	Percent Recovery
100 copies / µL	30.98	30.99	99%
10 copies / µL	34.29	34.29	100%
1 copy / µL	37.52	37.46	100%