Sera-Xtracta
Virus/Pathogen Kit

User guide

cytiva.com

29513970 AA

1 Introduction

Product Code

29506009

Important

Read the instructions carefully before using the products.

Intended use

The product is intended for Research Use Only and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes. Do not use internally or externally in humans or animals.

Safety

For use, handling and disposal of the product, refer to the Safety Data Sheets. Good laboratory practices should be followed at all times.

Storage

All kit components should be stored at room temperature (15°C–30°C). Once reconstituted, store Proteinase K at 2°C–8°C.

Expiry

For expiry date, refer to outer packaging label.

Reconstituted Proteinase K solution is stable for at least 4 months when stored at 2°C–8°C.

2 Components

Kit Components

Pack size: Standard pack for 96 extractions

Product code: 29506009

Component	Amount
Bead / Lysis Reagent	60 mL
Wash buffer	100 mL
SeraSil-Mag™ 400 beads	1.1 mL
SeraSil-Mag 700 beads	1.1 mL
Proteinase K	30 mg or 60 mg (lyophilised)

Materials to be supplied by the user

Chemicals:

- Ethanol (absolute)
- Nuclease free water

Equipment:

- 1.5–2.0 mL microtubes, DNase / RNAse-free.
- Laboratory heating block (thermoblock), for 1.5–2.0 mL microtubes.
- Vortex Mixer
- Bench-top centrifuges for 1.5–2.0 mL microtubes.
- Magnetic separation racks, suitable for 1.5–2.0 mL microtubes.
- Pipette tips with aerosol barrier

Note: All tubes and pipette tips should be DNase / RNase free grade. The working environment should also be subject to cleaning procedures to minimize the presence of extraneous DNases, RNases and other DNA / RNA material originating from operators, samples and other biological sources.

3 Description

Cytiva has developed a kit incorporating its magnetic bead based technology (Sera-Xtracta™ Virus/Pathogen Kit), for the isolation of viral/bacterial nucleic acids (RNA and DNA). The Sera-Xtracta Virus/Pathogen Kit has been optimised for the isolation of nucleic acid from swab samples collected into Primestore MTM (Longhorn Vaccines and Diagnostics LLC).

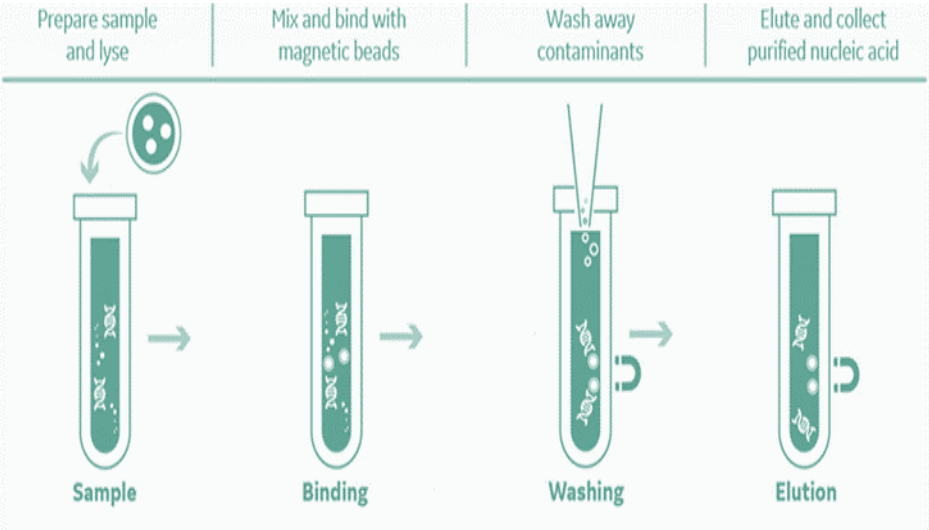
Infectious diseases affect millions of people every year. Particularly virulent and multi-drug resistant agents are increasingly responsible for infections with ever-expanding complexities. Molecular diagnostic laboratories and their test developers need to design, manufacture, and validate assays for these pathogenic agents. This requires the successful purification of high-quality nucleic acid which is essential to any molecular research and testing workflow. The nucleic acid purification process can be a bottleneck because sufficient nucleic acid from biological samples is required to meet a sensitivity threshold for an assay or tests which are designed to help mapping or making informed decisions on latent and active infections.

To address this bottleneck, Cytiva has developed a robust extraction chemistry

optimized with SeraSil-Mag magnetic beads for total nucleic acid (DNA/RNA) from various pathogen types. Our optimized reagent chemistry ensures total nucleic acid is selectively bound to the superparamagnetic SeraSil-Mag bead while impurities are efficiently removed during the quick wash steps. The resulting high quality total nucleic acid is then eluted from the beads. The rapid workflow (figure 1) is optimized for processing 100–400 µL of transport media to yield nucleic acid suitable for sensitive molecular testing including quantitative polymerase chain reaction (qPCR, RT-qPCR), droplet digital PCR (ddPCR), and next-generation sequencing (NGS). The kit contains enough reagents for 96 extractions (Product code: **29506009**)

3.1 Basic principle

Fig 1 Sera Xtracta Virus/Pathogen kit workflow.



Step procedure

Step	Comments	Component
1 Sample binding/lysis step	Cells are lysed by a chaotropic salt and detergent in the lysis buffer, in the presence of Proteinase K and nucleic acids are bound to the magnetic beads	Lysis Buffer Proteinase K SeraSil-Mag 400 beads SeraSil-Mag 700 beads
2 Wash 1 (buffer)	Wash Buffer containing a chaotropic salt removes contaminants from bound nucleic acids	Wash Buffer
3 Wash 2 (ethanol solution)	Further washing with ethanol to remove remaining contaminants	80% ethanol
4 Drying	Excess ethanol is removed by air drying	–
5 Elution	Nucleic acid is eluted in nuclease free water and aspirated away from the magnetic beads into a fresh tube	Nuclease free water

3.2 Product specifications

The Sera-Xtracta Virus/Pathogen Kit has been optimized for the isolation of nucleic acid from swab samples collected into Primestore MTM. For expected yields from other transport media please refer to paragraph 5.4.

Sample type	Swab sample collected into Primestore MTM
Sample input volume	100–400 µL
Elution volume	50 µL[1]
Yield	Detection of 1 copy/µL in Real-Time RT-PCR[2]

[1] Recommended elution volume: the end user has the option to vary this according to downstream application requirements

[2] Based on the concentration of synthetic viral RNA in the input sample

4 Protocol

4.1 Reagent preparation before use of kit

Proteinase K

Reconstitute Proteinase K to 20mg/mL with nuclease-free water (for example 1.5 mL added to 30 mg vial). Reconstituted enzyme is stable for at least 4 months when stored at 2°C to 8°C.

80% ethanol wash solution (Wash 2)

Prepare an 80% ethanol wash solution. **Note**: Prepare enough 80% Ethanol for 950 µL per extraction reaction. Use 100% absolute ethanol and nuclease-free water.

SeraSil-Mag bead working solution

Prepare a working solution of SeraSil-Mag 400 and SeraSil-Mag 700 beads (supplied as separate vials in the kit) in a 1:1 ratio. Vortex SeraSil-Mag beads thoroughly before each is added to the pre-mixture and then again prior to use. **Note:** Prepare sufficient bead volume for 20 µL bead mixture per reaction.

Elution

Pre-heat an aliquot of nuclease-free water for final nucleic acid elution from the beads, using a heated incubator set at 70°–75°C. **Note**: typical elution volume is 50 µL per reaction.

4.2 Protocol for nucleic extraction from 100–400 µL sample

Step 1: Lysis and Nucleic Acid Binding

1. Add 10 µL of **Proteinase K** Solution to a 1.5–2.0 mL microcentrifuge tube.
2. Add 100–400 µL of sample to the tube.
3. Add 20 µL of **SeraSil-Mag bead working solution** (as prepared above) and mix the solution by slowly pipetting up/down 5–10 times **Note**: Prior to adding, ensure SeraSil-Mag bead tubes are thoroughly vortexed and mix the beads frequently during pipetting.
4. Add 570 µL of **Binding/Lysis Reagent** to the 1.5–2.0 mL microcentrifuge tube. **Note**: Solution is highly viscous; pipette slowly to avoid a void volume in the tip and excess foaming.
5. Ensure thorough mixing of the solution, cap and place tube on vortex mixer set a medium speed for 1 min. Pulse spin contents in a microcentrifuge to bring contents down.
6. Incubate tube on heat block set to 70°C–75°C for 3 min. **Note**: Heat step enhances lysis and activates Proteinase K enzyme. If precipitation is evident, quick spin tube to bring down contents.
7. Place tube on magnet stand for 1 min or until the solution becomes clear. Without disturbing bound beads, carefully remove the entire supernatant.

Step 2: Wash the Bound RNA/DNA

1. Remove the sample tube from the magnet stand, add 950 µL **Wash Buffer (Wash 1)** and mix the solution by slowly pipetting up/down 5–10 times.
2. Ensure thorough mixing of the solution, cap and place tube on vortex mixer set a medium speed for 1 min. Pulse spin contents in a microcentrifuge to bring contents down.
3. Place the tube on a magnet stand for 1 min or until the solution becomes clear. Without disturbing bound beads, carefully remove the entire supernatant.
4. Remove the tube from the magnet stand and add 950 µL of freshly prepared **80% ethanol (Wash 2)** to the sample tube.
5. Mix tube contents by slowly pipetting the contents up/down 5–10 times. Pulse spin contents in a microcentrifuge to bring contents down.
6. Place the tube on the magnet stand for 1 minute or until the solution becomes clear. While on the magnet stand, carefully remove the supernantant without disturbing the pellet.
7. Briefly remove the tube from the magnet stand allowing the beads to sink towards the bottom of the tube. Note: This should take 3–5 s.
8. Place the tube back onto the magnet. When the beads collect to the magnet use a 10 or 20 µL pipette to carefully remove any remaining ethanol.
   Important: It is important to ensure that all the ethanol is removed.

Step 3: Elution of RNA/DNA

1. Add 50 µL of pre-heated (70°C–75°C) **nuclease-free water** to each sample tube. Pipette up and down slowly until all the beads are removed from the side of the tube and the entire bead mass is at the bottom of the tube (pulse spin the tubes in a microcentrifuge if needed)
2. Place the tube on the magnet stand for 1 min or until contents are clear.
3. With the tube on the magnet, carefully transfer the eluate, containing the extracted RNA/DNA sample to a new microcentrifuge tube.

Notes;

A pulse spin in a microfuge is strongly recommended before magnet settling to ensure all the liquid sample in the tube is collected together in a single bulk volume at the bottom of the tube. Isolated droplets on the tube walls or trapped under the tube lid will affect results.

4.3 Storage of recovered nucleic acid

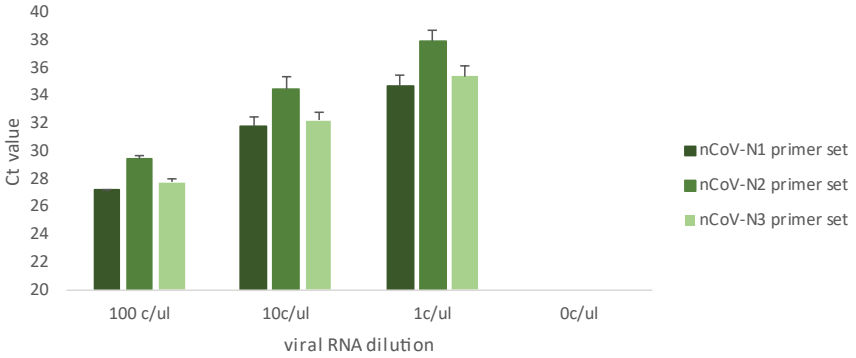
The protocol recommends elution of the sample in nuclease free water. Purified DNA or RNA maybe stored at 2°C–8°C for a short period when used immediately for analysis and / or downstream molecular biology / analytical applications. For long term storage aliquot and store purified DNA isolates at -20°C and RNA isolates at -80°C or less (user might consider using standard TE buffer, pH. 8–8.5 for DNA or 1 mM sodium citrate, pH 6.5 for RNA).

5 Appendices

For all experiments, the recovery of nucleic acid has been demonstrated using Real-Time RT-PCR assay, performed 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 Covid-19 nucleocapsid gene and RNase P primers targeting human RNase P gene, Integrated DNA Technologies).

5.1 Recovery of synthetic SARS-CoV-2 RNA

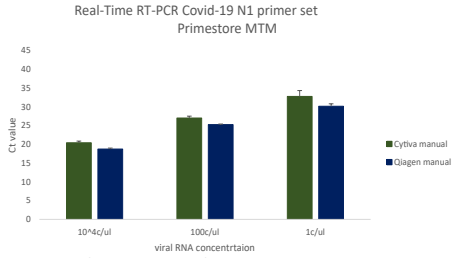
Experiments were carried out to test the extraction of a synthetic SARS-CoV-2 RNA (N gene, Seracare) spiked into a diluent consisting of a suspension of human cells (~3 x 10^5/mL) in 400 µL Primestore MTM (FDA approved transport media) to mimic a clinical sample. Averages of between 2 and 6 replicates per condition were obtained from multiple experiments, by multiple operators using two batches of the extraction kit reagents. Cytiva Sera-Xtracta Virus/Pathogen kit allows for confident detection of viral RNA down to 1 copy/µL in the input sample. For comparison with the CDC published data for the 2 referenced QIAGEN kits please refer to the table below (CDC Division of Viral Diseases, CDC-006-00019).

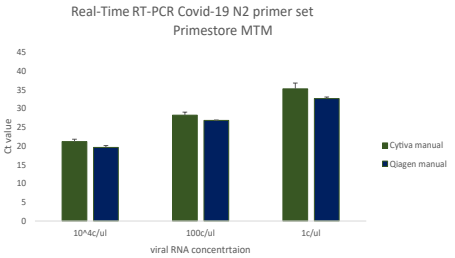


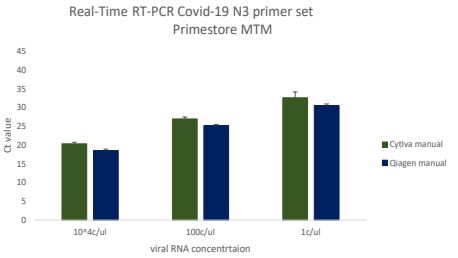
RNA Target Conc 1 copy/µL in MTM	nCoV-N1	nCoV-N2	nCoV-N3
Cytiva Sera-Xtracta Virus/Pathogen kit (Primestore MTM, 400 µl)	**34.6**	**37.9**	**35.4**
QIAGEN QIAmp DSP Viral RNA Mini kit (VTM identity and volume not disclosed)	32.8	35.4	32.7
QIAGEN EZ1 DSP Virus kit (VTM identity and volume not disclosed), LOD > 3.16 copies/µL	35.4	–	–

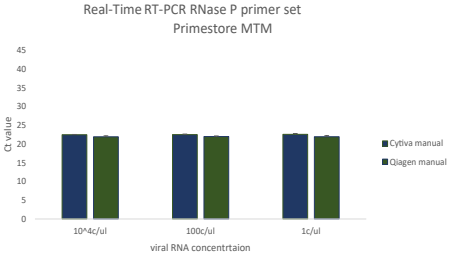
5.2 Comparative performance of Sera-Xtracta Virus/ Pathogen kit

We have compared the performance of the Sera-Xtracta Virus/Pathogen kit for the extraction of a synthetic SARS-CoV-2 RNA (N gene, AB Scientific) spiked at between 1 and 10 000 copies/µL into a diluent consisting of a suspension of human cells (~3 x 10^5/mL) in 200 µL of Primestore MTM to mimic a clinical sample with that of one of the CDC recommended extraction kits (QIAGEN QIAamp MiniElute Virus Spin kit) for COVID-19 virus detection (as per manufacturer's instructions). Data from 3 independent experiments are presented below and confirm that both kits are able to detect the presence of viral RNA down to 1 copy/µL in the input sample.



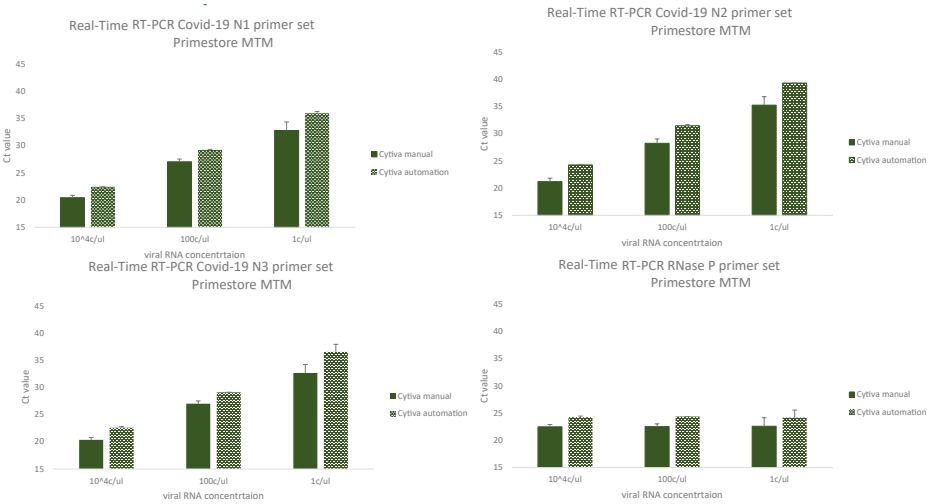






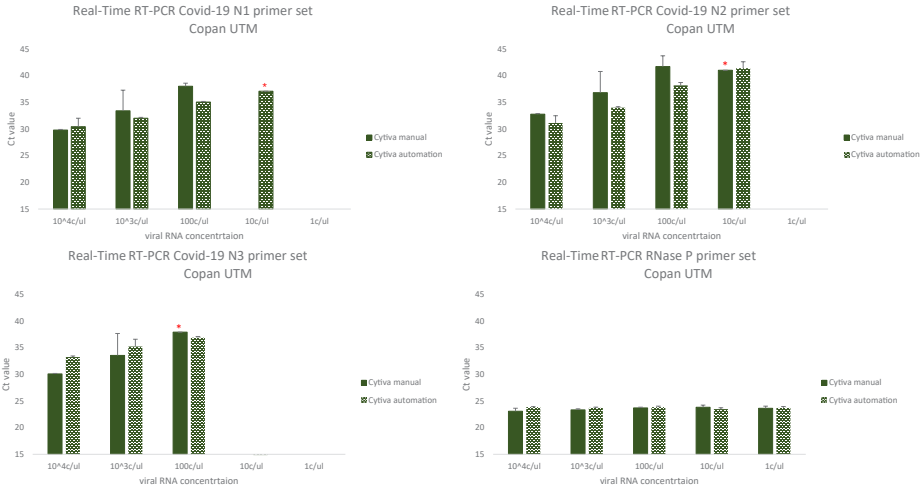
5.3 Use of an automated protocol

We have assessed the use of the Sera-Xtracta Virus/Pathogen kit in an automated system (Kingfisher DUO, Thermo Scientific Inc). Experiments were carried out to test the extraction of a synthetic SARS-CoV-2 RNA (N gene, AB Scientific) in 200 µL of the Primestore MTM transport media containing between 1 and 10 000 copies/µL of viral RNA in the presence of human cells as described previously. Data from 2 independent experiments from both manual and automated extraction method are shown below and confirm that both methods enable detection of the viral RNA down to 1copy/µl in the input sample. For further information, the automation script description is available upon request.



5.4 Use with other UTM / VTM media

Experiments were carried out to compare the extraction of a synthetic viral RNA from an alternative UTM using both manual and automated method (Kingfisher DUO). 200 µL of Copan UTM (Becton Dickinson) containing human cells (~3 x 10^5/mL) was spiked with a synthetic SARS-CoV-2 RNA (N gene, AB Scientific) at between 1 and 10 000 copies/µL and processed as described before. Data from 2 independent experiments are presented below and confirm successful detection of the viral RNA load down to 100 copies/µl in the input sample independently of the method used (automation and manual) when using alternative transport media.



* Not all replicates successfully detected

6 Related products

Product	Pack size	Product code
Sera Xtracta Cell-Free DNA Kit	96 purifications (2ml input)	29437807
Sera Xtracta Genomic DNA Kit	96 purifications	29429140
RNAspin Mini Kit	20 preps 50 preps 250 preps	25050070 25050071 25050072
RNAspin 96 Kit	4 x 96 preps	25050075
Sera-Mag Select	5 mL 60 mL 450 mL	29343045 29343052 29343057
PuRe Taq Ready-To-Go PCR beads	Multiwell plate, 96 reactions Multiwell plate, 5 x 96 reactions 0.5 mL tubes, 100 reactions 0.2 mL hinged tube with cap, 96 reactions	27955701 27955702 27955801 27955901
GenomiPhi V2 DNA amplification kit	100 reactions 500 reactions	25660031 25660032
Ready-To-Go GenomiPhi V3 DNA amplification kit	10 purifications 100 purifications 200 purifications	28903466 28903470 28903471
GFX 96 PCR Purification Kit	96 purifications	28903445
Blood genomicPrep Mini Spin Kit	10 purifications 50 purifications 250 purifications	10 purifications 50 purifications 250 purifications
Tissue and Cells genomicPrep Mini Spin Kit	50 purifications 250 purifications	28904275 28904276
MagRack Maxi	15 mL / 50 mL tubes	28986441
MagRack 6	1.5mL / 2.0 mL microtubes	28948964

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29513970 AA 04/2020