Sera-Xtracta Virus/Pathogen Kit

Quick-start user guide

Product description

Sera-Xtracta™ Virus/Pathogen Kit is intended for Laboratory 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. For use and handling and disposal of the product, refer to the Safety Data Sheets. Good laboratory practices should be followed at all times.

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 bead. The protocol is optimized for the isolation of nucleic acid from swab samples collected into Primestore MTM (Longhorn Vaccines and Diagnostics LLC). Processing 100–400 µL of transport media yields 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**) or 1000 extractions (Product code: **29514201**).

Kit components

	96 extractions	1000 extractions
Binding/Lysis Reagent	60 mL	630 mL
Wash Buffer	100 mL	1000 mL
SeraSil-Mag 400 beads	1.1 mL	10.4 mL
SeraSil-Mag 700 beads	1.1 mL	10.4 mL
Proteinase K liquid	1.5 mL	2 × 5.5 mL

All kit components should be stored at room temperature (15°C–30°C).
For expiry date, refer to outer packaging label.

Required additional chemicals and equipment

- Ethanol (absolute)
- Nuclease free water
- 1.5–2.0 mL microtubes, ideally sterile, 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

Reagent preparation before use of kit

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°C–75°C. **Note:** Typical elution volume is 50 µL per reaction.

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 minute. Pulse spin contents in a microcentrifuge to bring contents down.
6. Incubate tube on heat block set to 60°C for 10 minutes. **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 minute 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 and add 950 µL Wash Buffer (Wash 1) to the sample tube. Mix by slowly pipetting the contents 5–10 times.
2. Ensure thorough mixing of the solution, cap and place tube on vortex mixer set a medium speed for 1 minute. Pulse spin contents in a microcentrifuge to bring contents down.
3. Place the tube on a magnet stand for 1 minute 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 supernatant 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 seconds.
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
9. Remove tube from magnetic stand and allow beads to dry for 2 minutes.

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 minute 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.

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, the user might consider using standard TE buffer, pH 8–8.5 for DNA or 1 mM sodium citrate, pH 6.5 for RNA.

cytiva.com

CY12599-30Nov20-QA

cytiva