Maxwell® RSC Viral Total Nucleic Acid Purification Kit

Instructions for Use of Products AS1330 and ASB1330.

Promega

Quick Protocol

Collection and Storage of Samples Before Purification

Blood-borne pathogen precautions are recommended when handling any human-derived specimens. Collect blood in EDTA- or ACD-anticoagulated Vacutainer® tubes. Avoid heparin as it may inhibit downstream amplifications. Separate plasma from cells within 1 hour of drawing blood by centrifuging at 1,500 × *g* for 20 minutes at 25°C, then decant into a clean tube. Separate serum from clotted blood by centrifuging at 1,000 × *g* for 10 minutes at 25°C, then decant into a clean tube. Store plasma and serum samples at 2–8°C for up to 24 hours, or freeze samples that are not processed within 24 hours at –20°C for up to 5 days. Avoid repeated freeze-thaw cycles, and do not store samples in a frost-free freezer. Specific collection and storage conditions may vary, depending on the virus isolated.

Purification of Total Viral Nucleic Acid from Plasma or Serum

Maintain an RNase-free environment during processing. Always use RNase-free and aerosol-resistant pipette tips. Change gloves frequently to reduce the chance of RNase contamination.

Materials to Be Supplied by the User

- 1.5ml or 2.0ml microcentrifuge tubes, nuclease-free
- tube for Lysis Solution
- heat block or water bath set to 56°C
- RNase-free, sterile, aerosol-resistant pipette tips

Preparation of Lysis Solution

Prepare fresh Lysis Solution for each batch of samples as described in the following table. We recommended preparing approximately 20% extra Lysis Solution to compensate for potential pipetting losses.

For 100µl and 200µl plasma or serum samples:

Reagent	Volume for One Sample	Volume for 16 Samples[1]
Lysis Buffer[2]	200µl	3,800µl
Proteinase K	20µl	380µl

For 300µl plasma or serum samples:

Reagent	Volume for One Sample	Volume for 16 Samples[1]
Lysis Buffer[2]	300µl	5,700µl
Proteinase K	30µl	570µl

[1]The volumes listed for Lysis Buffer and Proteinase K Solution for 16 samples include approximately 20% extra volume.

[2]If an internal control is used, it may be added to the Lysis Solution. Internal controls are not provided in this kit.

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Preparation of Samples

Plasma or serum samples may be fresh or frozen. Thaw frozen specimens at room temperature or on ice, and mix by vortexing for 10 seconds before use.

1. Pipet each plasma or serum sample into a 1.5ml or 2ml microcentrifuge tube with a cap.
2. Add Lysis Solution prepared in previous section:
   a. To 100µl or 200µl samples, add 220µl of Lysis Solution.
   b. To 300µl samples, 330µl of Lysis Solution.
3. Close tubes, and vortex for 10 seconds.
4. For plasma samples, proceed to Step 5. For serum samples, incubate at room temperature (15–30°C) for 10 minutes, then proceed to Step 5.
5. Incubate at 56°C in a heat block or water bath for 10 minutes. During this incubation, proceed to next section to prepare the cartridges.
   Note: Samples containing virus such as hepatitis B virus require incubation at 80°C for optimal nucleic acid recovery due to secondary structure of the viral genome.

Maxwell® Automated Purification

Cartridge Preparation

1. Place the cartridges to be used in the Deck Tray(s) with well #1 (the largest well) facing away from the elution tube.
2. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
3. Place a plunger in well #8 of each cartridge. Well #8 is the well closest to the elution tube.
4. Place elution tubes in the front of the Deck Tray. Add 50µl of Nuclease-Free Water to the bottom of each elution tube.
   Notes:
   1. If Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.
   2. Use only the Elution Tubes (0.5ml) provided in the kit; other tubes may be incompatible with supported Maxwell® Instruments.
   3. The elution volume may require optimization for downstream applications. The recommended elution volume for the Maxwell® RSC Viral Total Nucleic Acid Purification Kit is 50µl of Nuclease-Free Water.
5. Transfer sample lysate to well #1 (the largest well) of the cartridge.

Instrument Run on the Maxwell® Instruments

Follow the instrument run instructions in the *Maxwell® RSC Viral Total Nucleic Acid Purification Kit Technical Manual* #TM420.

Additional protocol information is in Technical Manual #TM420, available online at: **www.promega.com**

