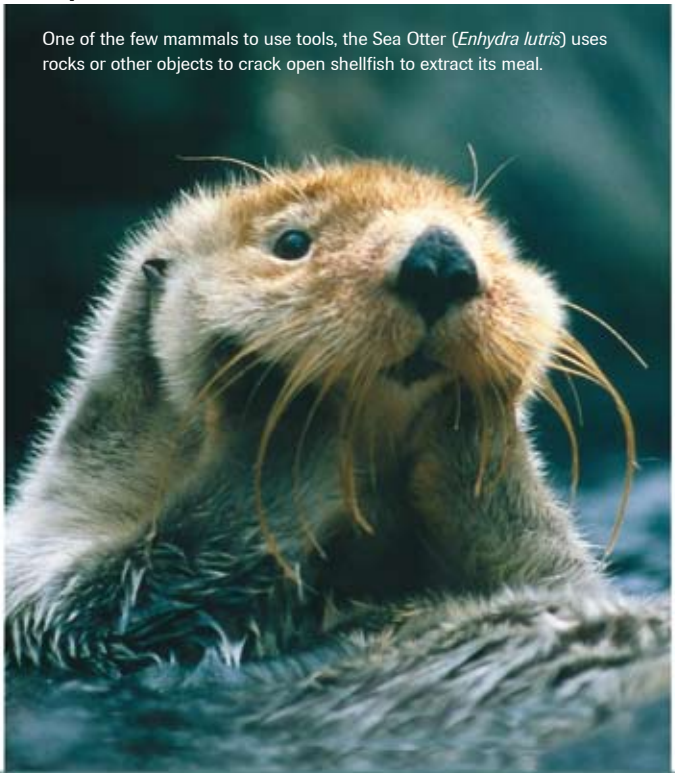
High Pure Viral Nucleic Acid Kit

Rapidly isolate highly purified viral DNA and RNA

Choose the **High Pure Viral Nucleic Acid Kit** to efficiently isolate viral DNA and RNA from a broad range of research sample materials, including:
Serum, plasma, whole blood, cell culture supernatant, peripheral blood mononuclear cells (PBMCs), cerebrospinal fluid (CSF), tongue scrapings, and throat wash samples.

Use a simple, rapid protocol to generate high-quality templates for direct use in PCR, RT-PCR, qPCR, and qRT-PCR applications.

Experts at Extraction



One of the few mammals to use tools, the Sea Otter (*Enhydra lutris*) uses rocks or other objects to crack open shellfish to extract its meal.

■ Use one versatile kit for diverse applications.

Isolate viral DNA and RNA from a wide variety of sample materials, enabling simultaneous analysis of both virus types.

■ Save time with a rapid, easy-to-use protocol.

Prepare multiple PCR/RT-PCR templates in 20 minutes, with only 10 minutes of hands-on time.

■ Maximize performance and accuracy in downstream assays.

Obtain highly pure, concentrated (50 µl) nucleic acids that provide high sensitivity, reproducibility, and specificity in real-time PCR and other applications (Figure 1).

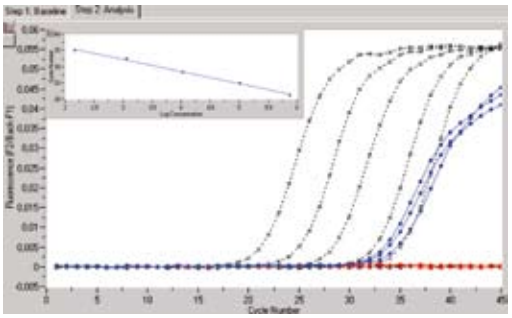


Figure 1: Real-time, on-line PCR detection for the quantification of Epstein-Barr Virus (EBV) DNA in research samples. EDTA blood samples, artificially spiked with quantified EBV virus material, were extracted using the High Pure Viral Nucleic Acid Kit and analyzed using the LightCycler® EBV Quantification Kit in qPCR on a LightCycler® Instrument. DNA standards ranging from 10^6 to 10^2 copies/reaction (black line with crosses) were used to generate a standard curve.
EBV-positive blood samples (triplicate, spiked with the EBV genome containing Namalwa cell line) correspond to the lower limit of 400 copies/reaction (blue line with dots). The negative control (blood not spiked with Namalwa cell line) is indicated by the red line with dots.
Results: Efficient nucleic extraction using the High Pure Viral Nucleic Acid Kit generates purified nucleic acids that do not inhibit the amplification reaction.

Efficiently purify viral nucleic acids

Procedure

Protocol for isolating viral nucleic acids from 200 µl serum, plasma, or whole blood.

View detailed procedures for other sample materials in the pack insert at **www.roche-applied-science.com**

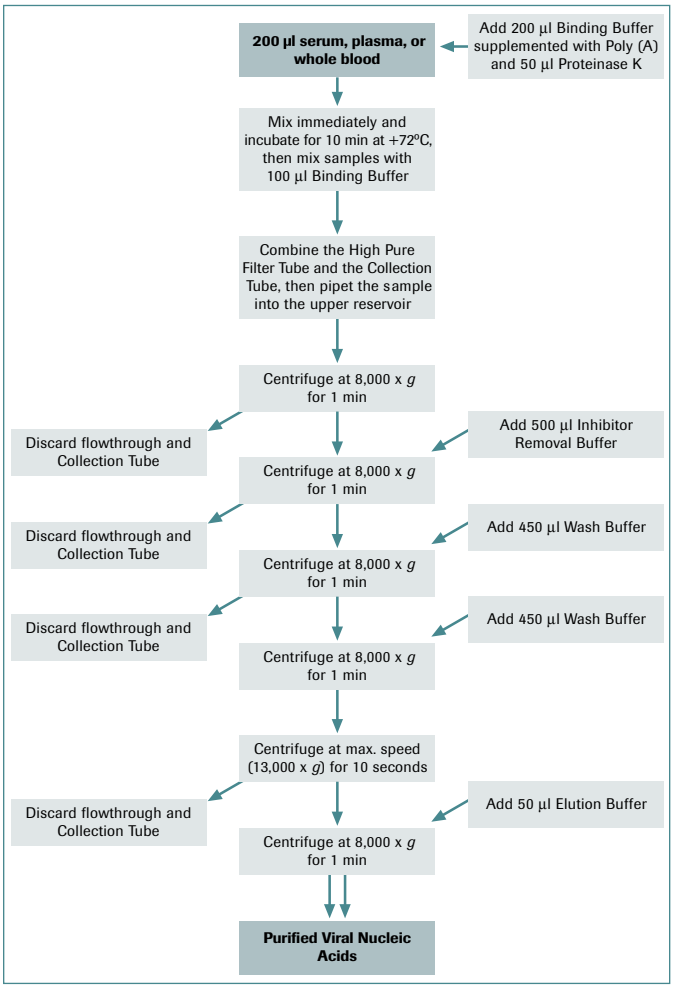
Note: For the isolation of nucleic acids from whole blood, pre-warm the elution buffer to +70°C.

1. To a nuclease-free 1.5 ml microcentrifuge tube:
   - Add 200 µl serum, plasma, or whole blood.
   - Add 200 µl working solution, freshly prepared (carrier RNA-supplemented Binding Buffer).
   - Add 50 µl Proteinase K solution; mix immediately.
   - Incubate for 10 min at +72°C.
2. Add 100 µl Binding Buffer and mix well.
3. To transfer the sample to a High Pure Filter Tube:
   - Insert one High Pure Filter Tube into one Collection Tube.
   - Pipet the entire sample into the upper reservoir of the Filter Tube.
4. - Insert the entire High Pure Filter Tube assembly into a standard table-top centrifuge.
   - Centrifuge 1 min at 8,000 × *g*.
5. After centrifugation:
   - Remove the Filter Tube from the Collection Tube; discard the flowthrough and the Collection Tube.
   - Combine the Filter Tube with a new Collection Tube.
6. After combining the Filter Tube with a new Collection Tube:
   - Add 500 µl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube.
   - Centrifuge 1 min at 8,000 × *g*.
7. After centrifugation:
   - Remove the Filter Tube from the Collection Tube; discard the flowthrough and the Collection Tube.
   - Combine the Filter Tube with a new Collection Tube.
8. After removal of inhibitors:
   - Add 450 µl Wash Buffer to the upper reservoir of the Filter Tube.
   - Centrifuge 1 min at 8,000 × *g* and discard the flowthrough.
9. After the first wash and centrifugation:
   - Remove the Filter Tube from the Collection Tube; discard the flowthrough and the Collection Tube.
   - Combine the Filter Tube with a new Collection Tube.
   - Add 450 µl Wash Buffer to the upper reservoir of the Filter Tube.
   - Centrifuge 1 min at 8,000 × *g* and discard the flowthrough.
   - Leave the Filter Tube-Collection Tube assembly in the centrifuge and spin it for 10 s at maximum speed (approx. 13,000 × *g*) to remove any residual Wash Buffer.

   ! The extra centrifugation time ensures removal of residual Wash Buffer.
10. Discard the Collection Tube and insert the Filter Tube into a nuclease-free, sterile 1.5 ml microcentrifuge tube.
11. To elute the viral nucleic acids:
    - Add 50 µl Elution Buffer to the upper reservoir of the Filter Tube.
    - Centrifuge the tube assembly for 1 min at 8,000 × *g*.
12. The microcentrifuge tube contains the eluted, purified viral nucleic acids.

    ! Use the eluted nucleic acids directly in PCR (10 – 20 µl DNA eluate) or RT-PCR (3.5 µl viral RNA); or, store the eluted viral RNA at –80°C or the viral DNA at +2 to +8°C or –15 to –25°C for later analysis.

High Pure Viral Nucleic Acid Kit workflow



Ordering information

Product	Cat. No.	Pack Size
High Pure Viral Nucleic Acid Kit	11 858 874 001	Up to 100 isolations

For more information about the **High Pure Viral Nucleic Acid Kit** and other products for nucleic acid isolation and purification, visit
www.roche-applied-science.com/napure

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