USER GUIDE

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PureLink® Viral RNA/DNA Kits

For rapid, efficient purification of viral nucleic acids from cell-free samples

Catalog Numbers 12280-050, 12280-096

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Contents

Experienced Users' Mini Kit Procedure

Introduction

This quick reference sheet is included for experienced users of the PureLink® Viral RNA/DNA Mini Kit. If you are a first time user, follow the detailed protocol in this manual.

Step	Action
Preparing Lysates	The lysate preparation protocol is described below for **200 µL** starting material. If you wish to process >200 µL (≤500 µL) sample volume, scale-up the reagent volumes accordingly. 1. Add 25 µL Proteinase K into a sterile microcentrifuge tube. 2. Add 200 µL of cell-free sample into the microcentrifuge tube. **Note:** If you are processing <200 µL sample, adjust final volume of the sample to 200 µL using PBS (phosphate buffered saline) or 0.9% NaCl. 3. Add 200 µL Lysis Buffer (containing 5.6 µg Carrier RNA). Close the tube lid and mix by vortexing for 15 seconds. 4. Incubate at 56°C for 15 minutes. 5. Add 250 µL 96–100% ethanol to the tube, close the lid, and mix by vortexing for 15 seconds. 6. Incubate the lysate for 5 minutes at room temperature.
Purification Procedure	1. **Add** above lysate to the Viral Spin Column in a collection tube. 2. Centrifuge the column at 6800 × *g* for 1 minute. Discard the collection tube. Place the spin column in a new Wash Tube. 3. **Wash** the column with 500 µL Wash Buffer (W5) with ethanol. Centrifuge at 6800 × *g* for 1 minute. Discard the flow through. 4. **Repeat** wash Step 3 with 500 µL Wash Buffer (W5) once. 5. Discard the collection tube and place the spin column in another, clean Wash Tube. 6. Centrifuge the spin column at maximum speed for 1 minute to remove any residual Wash Buffer (W5). 7. Place the spin column in a clean 1.7-mL Recovery Tube. 8. **Elute** with 10–50 µL sterile RNase-free water (E3) supplied with the kit (add water to the center of the cartridge). 9. Incubate at room temperature for 1 minute. Centrifuge the spin column at maximum speed for 1 minute to elute nucleic acids. *The Recovery Tube contains purified viral nucleic acids*. Discard the spin column. 10. Store purified viral RNA/DNA at –80°C or use RNA/DNA for the desired downstream application.

Experienced Users' 96 Kit Procedure

Introduction

This quick reference sheet is included for experienced users of the PureLink® 96 Viral RNA/DNA Kit. If you are a first time user, follow the detailed protocol in this manual.

Step	Action
Preparing Lysates	The lysate preparation protocol is described below for **200 µL** starting material. 1. Add 25 µL Proteinase K to each well. 2. Add 200 µL of cell-free sample to the well. **Note:** If you are processing <200 µL sample, adjust final volume of the sample to 200 µL using PBS (phosphate buffered saline) or 0.9% NaCl. 3. Add 200 µL Lysis Buffer (containing 5.6 µg Carrier RNA). Mix by pipetting up and down 3–5 times. 4. Incubate at 56°C for 15 minutes. Briefly centrifuge. 5. Add 250 µL 96–100% ethanol to each well and mix by pipetting up and down 3–5 times. 6. Incubate the lysate for 5 minutes at room temperature.
Purification Procedure with Centrifu-gation	Perform all centrifuge steps at 25°C. 1. Transfer each lysate to the 96-well Filter Plate. Cover unused wells with foil tape. 2. Place the 96-well Filter Plate onto a 96-well, Deep-Well Plate and centrifuge at ≥2250 × *g* for 5 minutes. 3. Remove the flow-through and place the Filter Plate back on the Deep-Well Plate. 4. Add 500 µL Wash Buffer (W5) with ethanol. 5. Centrifuge 5 minutes at ≥2250 × *g* until liquid passes through the Filter Plate. 6. Remove the flow-through and place the Filter Plate back on the Deep-Well Plate. 7. Repeat Steps 4–6. 8. Centrifuge the Filter Plate at ≥2250 × *g* for 10 minutes to dry. 9. Add 100 µL RNase-free water and incubate the plate for 1 minute at room temperature. 10. Place the Filter Plate onto a Receiver Plate. Centrifuge at ≥2250 × *g* for 5 minutes. 11. Use the RNA/DNA for the desired downstream application. To store the purified RNA/DNA, cover the wells with foil tape, and store at –80°C.

Experienced Users 96 Kit Procedure,
Continued

Step	Action
Purification Procedure with Vacuum	1. Place a 96-well Filter Plate in top half of a vacuum manifold. 2. Transfer lysates from Preparing Lysates, Step 6 to the Filter Plate. Cover unused wells with foil tape. 3. Apply vacuum for 2 minutes at room temperature. Release. 4. Add 500 µL Wash Buffer (W5) with ethanol. 5. Apply vacuum 2 minutes at room temperature. Release. 6. Repeat Steps 4 and 5. 7. **Tap the Filter Plate on a stack of paper towels and pat dry.** 8. Return the Filter Plate to the vacuum manifold and apply vacuum for 10 minutes at room temperature. Release. 9. Replace the waste collection tray with the Receiver Plate and place the Filter Plate on top of the Receiver Plate. 10. Add 150 µL sterile, RNase-free water (E3) and incubate the plate for 1 minute at room temperature. 11. Apply vacuum for 2 minutes at room temperature. Release. 12. The RNA/DNA is eluted into the Receiver Plate in a volume of 120–130 µL. Use the RNA/DNA for the desired downstream application. To store the purified RNA/DNA, cover the wells with foil tape, and store at –80°C.

Kit Contents and Storage

Shipping and Storage

All components of the PureLink® Viral RNA/DNA Kits are shipped at room temperature.

Upon receipt, store all kit components at room temperature, **except store Carrier RNA at –20°C.**

Note: The Proteinase K solution is stable for 1 year when stored at room temperature. For long-term storage (>1 year) or if room temperature is >25ºC, store the Proteinase K solution at 4ºC.

Types of Kits

This manual supports the following kits.

Kit	Reactions	Catalog No.
PureLink® Viral RNA/DNA Mini Kit	50	12280-050
PureLink® 96 Viral RNA/DNA Kit	384	12280-096

Contents

The components and amounts included in the PureLink® Viral RNA/DNA Kits are listed in the following table.

Note: Since the Mini Kit is designed for purifying samples using a starting volume of ≤500 µL, some reagents in the kit maybe provided in excess in the amount needed. The 96 Kit sample volume is limited to 200 µL or less.

Component	12280-050	12280-096
Viral Lysis Buffer (L22)	32 mL	100 mL
Wash Buffer (W5) (5X)	15 mL	2 × 87.5 mL
Proteinase K (20 mg/mL) in storage buffer (proprietary)	2 × 1.6 mL	10 mL
Carrier RNA (lyophilized)	310 µg	2.2 mg
Sterile, RNase-free Water (E3)	15.5 mL	75 mL
Viral Spin Columns with Collection Tubes	50 each	—
Wash Tubes (2.0-mL)	2 × 50	—
Recovery Tubes (1.5-mL)	50 each	—
PureLink® Filter Plate	—	4 each
Receiver Plate	—	4 each
Deep-Well Plate	—	6 each
Foil Tape	—	3 × 4/bag

Introduction

Overview

Introduction

The PureLink® Viral RNA/DNA Kits provide a rapid and efficient method to simultaneously purify viral RNA/DNA from fresh or frozen cell-free biological fluids (plasma, serum, cerebrospinal fluid) and cell culture supernatants.

The PureLink® Viral Mini Kit is specifically designed to isolate high-quality viral nucleic acids from a variety of RNA and DNA viruses within 45 minutes using low elution volumes that allow sensitive downstream analysis.

The PureLink® 96 Viral Kit is specifically designed for obtaining the highest yield with samples of low titer. The kit allows high throughput isolation of viral nucleic acid using Tecan or Beckman instruments.

The purified viral RNA/DNA is devoid of proteins and nucleases, and is suitable for use in downstream applications that allow viral detection and genotyping.

System Overview

Both kits allow efficient lysis of viral particles at elevated temperatures using Proteinase K and selective binding of viral nucleic acids to the silica matrix under highly denaturing conditions.

The viral particles in the cell-free samples are lysed using Proteinase K and Lysis Buffer (L22) containing Carrier RNA at 56°C. The Lysis Buffer (L22) is specifically formulated to allow lysis of different types of viral particles.

Ethanol is added to the lysate to a final concentration of 37% and the sample is loaded onto a silica spin column (Mini Kit) or silica Filter Plate (96 Kit). The viral RNA/DNA molecules bind to the silica-based media and impurities such as proteins and nucleases are removed by thorough washing with Wash Buffer. The RNA/DNA is then eluted in sterile, RNase free water.

Advantages

The PureLink® Viral RNA/DNA Mini Kit provides the following advantages:

- Rapid and efficient purification of high-quality viral nucleic acid using spin column-based centrifugation with no sample cross-contamination
- Specifically designed to purify viral RNA and DNA from ≤500 µL cell-free samples within 45 minutes
- Ability to elute viral nucleic acids in low elution volumes of 10–50 µL to allow sensitive downstream analysis

In contrast, the PureLink® 96 Viral RNA/DNA Kit provides:

- Highest sensitivity for samples with very low viral titer
- High throughput processing of samples
- Automation using Tecan or Beckman robotic systems

Lastly, **both** kits provide:

- Purified nucleic acid free of contaminants such as proteins and nucleases
- Reliable performance of the purified viral nucleic acids in downstream applications

Carrier RNA

The Carrier RNA included with the PureLink® Viral RNA/DNA Kits is yeast tRNA (page 31). The presence of an excess amount of Carrier RNA as compared to viral nucleic acids during lysate preparation and purification:

- Increases the binding of viral nucleic acids to the silica matrix
- Reduces any viral nucleic acid degradation from nucleases present in the sample

The purification protocol recommends using 5.6 µg Carrier RNA for 200–500 µL of sample. Most of the Carrier RNA is removed during the purification process because it is <200 bp and any remaining Carrier RNA does not interfere with downstream applications. However, depending on your application, you may validate the assay using less Carrier RNA.

Overview, Continued

Proteinase K

The Proteinase K is used for efficient lysis of viral particles. Proteinase K is active in the highly denaturing conditions of the lysis step.

Downstream Applications

The purified viral RNA and DNA is suitable for use in RT-PCR, qRT-PCR, and qPCR, and can be used for:

- Viral load monitoring
- Viral detection
- Viral genotyping

Mini Kit Specifications

Starting Material:	≤500 µL cell-free sample
Binding Capacity:	~5 µg nucleic acid
Column Reservoir Capacity:	700 µL
Wash Tube Capacity:	2.0 mL
Recovery Tube Capacity:	1.5 mL
Centrifuge Compatibility:	Capable of centrifuging at >10,000 × *g*
Elution Volume:	10–50 µL

96 Kit Specifications

Dimensions:	Standard SBS (Society for Biomolecules Screening) footprint
Starting Material:	≤200 µL cell-free sample
Binding Capacity:	At least 40 µg nucleic acid
Filter Reservoir Capacity:	1.0 mL
Receiver Plate Capacity:	0.33 mL (0.1–0.2 mL)
Deep-Well Plate Capacity:	1.09 mL
Centrifuge Compatibility:	Capable of centrifuging at ≥2250 × g Bucket depth 5 cm
Elution Volume:	100–150 µL

Methods

Before Starting

Introduction

Review the information in this section before starting. Guidelines are included for the recommended amount of starting material for use and to obtain high-quality RNA.

WARNING! GENERAL CHEMICAL HANDLING. For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

The PureLink® Plant Total DNA Purification Kit buffers contain guanidine hydrochloride. Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid wastes containing this product.

Important

If there is any precipitate present in the buffers, warm the buffer up to 25°C to 37°C to dissolve the precipitate before use.

General Handling of RNA

Observe the following guidelines to prevent RNase contamination:

- Use disposable, individually wrapped, sterile plastic ware
- Use only sterile, new pipette tips (aerosol-barrier pipet tips recommended) and microcentrifuge tubes
- Wear latex gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin
- Always use proper microbiological aseptic techniques when working with RNA
- Use RNase *AWAY*® Reagent (page 31) to remove RNase contamination from surfaces

Before Starting, Continued

Starting Material

The PureLink® Viral RNA/DNA Kits are designed to isolate viral nucleic acid from cell-free biological fluids such as plasma, serum, and CSF (cerebrospinal fluid) as well as cell culture supernatant using fresh or frozen samples.

To obtain high yield of viral nucleic acids and minimize any degradation, follow these guidelines:

- Collect the sample (such as plasma or serum) and proceed immediately to the purification protocol (page 15 if using the Mini Kit; page 21 if using the 96 Kit). If desired, you can store the sample at 4ºC for short-term storage (≤4 hours) or freeze the sample at –20ºC or –80ºC for long-term storage.
- Do not freeze-thaw the plasma or serum sample more than once.
- Remove any visible cryoprecipitates from samples by centrifugation at ~7000 × *g* for 2–3 minutes. Use the clear supernatant immediately for purification.
- If you need to concentrate the cell culture supernatant use appropriate centrifugal concentrators.

Sample Volume

The PureLink® Viral RNA/DNA Mini Kit can process sample volumes of ≤500 µL without preparation of any additional buffers or carry over of any impurities. After preparing the lysate, if the final lysate volume is >600 µL, you need to perform multiple loadings of the final lysate onto the spin column.

The 96 Kit processes sample volumes of ≤200 µL.

Preparing Wash Buffer

For the Mini Kit:

Add 60 mL 96–100% ethanol to 15 mL Wash Buffer (W5) included with the kit.

For the 96 Kit:

Add 350 mL of 96–100% ethanol to 87.5 mL Wash Buffer (W5) included in the kit.

Place a check in the box on the Wash Buffer label to indicate the ethanol is added. Store the Wash Buffer (W5) with ethanol at room temperature.

Preparing Carrier RNA

The recommended purification protocol uses 5.6 µg Carrier RNA per sample (for ≤500 µL sample). If you wish to use less Carrier RNA per sample, you need to validate the amount of Carrier RNA needed for each sample type and downstream application.

To prepare Carrier RNA (5.6 µg/sample):

1. Add 310 µL RNase-free Water (included with the kit) to 310 µg lyophilized Carrier RNA supplied in a tube with the kit to obtain 1 µg/µL Carrier RNA stock solution.
2. Mix thoroughly and aliquot the solution into smaller aliquots. Store the aliquots at –20ºC. Avoid repeated freezing and thawing.
3. Calculate the volume of Lysis Buffer/Carrier RNA mix required to process the desired number of samples simultaneously using the following formula:

   N × 0.21 mL (volume of Lysis Buffer/reaction) = A mL

   A mL × 28 µL/mL = B µL

   where

   N = number of samples

   A = calculated volume of Lysis Buffer (L22)

   B = calculated volume of 1 µg/µL Carrier RNA stock solution to add to Lysis Buffer (L22)
4. Thaw the required amount of 1 µg/µL Carrier RNA stock solution.
5. In a sterile tube, add the volume of Carrier RNA stock solution (B, calculated as above) to the volume of Lysis Buffer (A, calculated as above). Mix gently by pipetting up and down. Avoid vortexing as it generates foam.
6. Store at 4ºC until use. **Use the buffer within 1 hour.**

Example:

The example below shows the amount of Lysis Buffer (L22) and Carrier RNA stock solution required to process **10** samples using the above formula:

10 × 0.21 mL (volume of Lysis Buffer/reaction) = 2.1 mL

2.1 mL × 28 µL/mL = 58.8 µL

To prepare Lysis Buffer containing Carrier RNA for processing 10 samples, mix 58.8 µL Carrier RNA stock solution with 2.1 mL Lysis Buffer (L22).

Instrument Compatibility for 96-Well Plates

The PureLink® Filter Plates are compatible with the following instruments:

- **Vacuum Manifold:** The manifold must accommodate the PureLink® Plates and be capable of collecting the filtrate (e.g., UniVac 3 Vacuum Manifold System from Whatman)
- **Centrifuge:** Must be capable of centrifuging 96-well plates obtaining ≥2250 × *g*, and accommodate a 5.0-cm microtiter plate stack.
- **Automated Liquid Handling Workstation:** The workstation must be equipped with a vacuum manifold and a vacuum source. The PureLink® Filter Plate has been tested and qualified for use on the Biomek® FX and the Tecan Freedom EVO™ Workstations.

  For the Biomek® FX, you may use the Small Vacuum Adapter Collar (Whatman Cat. no. 7705-0120) with PureLink® Plates.

The PureLink® Filter Plates are compatible with the QIAvac Vacuum Manifold, but **not** with the BioRobot® Workstations.

Calibrating Vacuum for Use with 96-Well Plates

We recommend using a vacuum pressure of –12 to –15 in. Hg to obtain the best results.

Using higher vacuum pressure than the recommended pressure may cause sample splattering or inefficient DNA binding, while using lower vacuum pressure will affect the elution resulting in lower recovery.

To check the vacuum pressure:

1. Place an unused PureLink® Filter Plate on top of the vacuum manifold.
2. Apply vacuum and check the vacuum pressure on the vacuum regulator (usually attached to the manifold or a vacuum pump).
3. Adjust the vacuum pressure on the regulator to obtain the recommended pressure of –12 to –15 in. Hg.

   Note: During purification the vacuum pressure may exceed the recommended value.

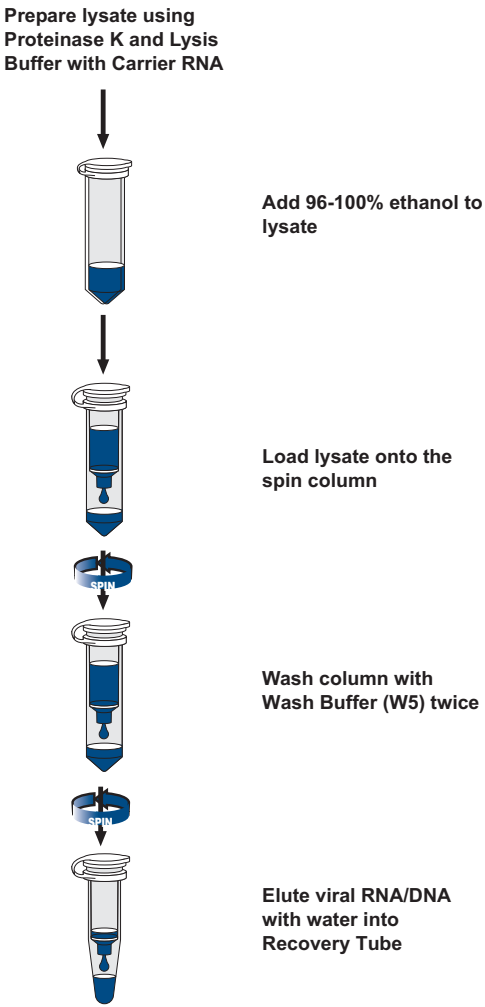
Purification Procedure for Mini Kit

Introduction

The viral nucleic acid purification procedure is described below using spin column based centrifugation in a total time of ~**45 minutes**.

Experimental Overview

The flow chart for purifying viral RNA/DNA using the PureLink® Viral RNA/DNA Mini Kit is shown below.



Purification Procedure for Mini Kit, Continued

Follow the recommendations below to obtain the best results:

- Perform all centrifugation steps at room temperature
- Be sure to add ethanol to Wash Buffer (W5) before use (page 12)
- Perform the recommended wash steps to obtain high-quality RNA
- Always pipet water in the center of the Viral Spin Column and perform a 1 minute incubation before elution

Important

- Handle all viruses in compliance with established institutional guidelines. Since safety requirements for use and handling of viruses may vary at individual institutions, we recommend consulting the health and safety guidelines and/or officers at your institution.
- Be sure to take the appropriate precautions (wear a laboratory coat, disposable gloves, and eye protection) when handling viral samples.
- The eluates collected during wash steps contain biohazardous waste. Dispose the eluate and collection tubes appropriately as biohazardous waste.

Elution Volume

The PureLink® Viral RNA/DNA Mini Kit utilizes low, recommended elution volume of 10–50 µL to elute viral nucleic acid resulting in highly concentrated viral nucleic acids that is required for sensitive downstream applications.

You may elute the viral nucleic acids in an elution volume ranging from 10–150 µL depending on your downstream applications.

Note: Using larger elution volume decreases the viral nucleic acid concentration in the eluate.

Purification Procedure for Mini Kit, Continued

Materials Needed

Components supplied by the user

- Cell-free samples (such as plasma or serum samples)
- Appropriate amount of Lysis Buffer (L22) containing Carrier RNA, page 13
- Heat block set to 56ºC
- 96–100% ethanol
- Sterile 1.5-mL or 2-mL microcentrifuge tubes
- Microcentrifuge capable of centrifuging >10,000 × *g*
- *Optional*: Sterile, RNase-free PBS or 0.9% NaCl

Components supplied with the Kit

- Wash Buffer (W5)
- Sterile, RNase-free Water (E3)
- Viral Spin Column in Collection Tubes
- Wash Tubes and Recovery Tubes

Preparing Lysate

The lysate preparation protocol is described below for **200 µL** starting material. If you wish to process >200 µL (≤500 µL) sample volume, scale-up the reagent volumes accordingly.

Note: There is no need to scale-up the amount of Carrier RNA. Use ≤5.6 µg Carrier RNA per sample volume of ≤500 µL.

1. Add 25 µL Proteinase K (included with the kit) into a sterile microcentrifuge tube.
2. Add 200 µL of cell-free sample (equilibrated to room temperature) into the microcentrifuge tube.

   Note: If you are processing <200 µL sample, adjust final volume of the sample to 200 µL using PBS or 0.9% NaCl.
3. Add 200 µL Lysis Buffer (containing 5.6 µg Carrier RNA). Close the tube lid and mix by vortexing for 15 seconds.
4. Incubate at 56ºC for 15 minutes.
5. Briefly centrifuge the tube to remove any drops from the inside of the lid.
6. Proceed immediately to **Binding and Washing Step**, page 18.

Binding and Washing Step

1. Add 250 µL 96–100% ethanol to the lysate tube to obtain a final ethanol concentration of 37%, close the lid, and mix by vortexing for 15 seconds.

   Note: If you are processing up to 10 samples, you may add ethanol to all tubes and then vortex each tube.
2. Incubate the lysate with ethanol for 5 minutes at room temperature.
3. Briefly centrifuge the tube to remove any drops from the inside of the lid.
4. Transfer the above lysate with ethanol (~675 µL) onto the Viral Spin Column.
5. Close the lid and centrifuge the column at ~6800 × *g* for 1 minute. Discard the collection tube with the flow-through.

   Note: If you are processing >200 µL starting material, you need to perform multiple loadings of the lysate by transferring any remaining lysate to the same Viral Spin Column and centrifuge at 6800 × *g* for 1 minute.
6. Place the spin column in a clean Wash Tube (2 mL) included with the kit and add 500 µL Wash Buffer (W5) with ethanol to the spin column.
7. Close the lid and centrifuge the column at ~6800 × *g* for 1 minute. Discard the flow-through and place the spin column back into the Wash Tube.

   Note: Additional Wash Tubes are available separately (page 31), if you do not wish to reuse the Wash Tube.
8. Add 500 µL Wash Buffer (W5) with ethanol into the spin column.
9. Close the lid, centrifuge at ~6800 × *g* for 1 minute. Discard the Wash Tube containing the flow-through.
10. Place the spin column in another clean, Wash Tube (2 mL) included with the kit.
11. Centrifuge the column at maximum speed in a microcentrifuge for 1 minute to dry the membrane completely. Discard the Wash Tube with the flow-through.
12. Proceed to the **Elution Step**, page 19.

Purification Procedure for Mini Kit, Continued

Elution Step

1. Place the Viral Spin Column in a clean 1.5-mL Recovery Tube supplied with the kit.
2. Add 10–50 µL of Sterile, RNase-free water (E3) to the center of the column. Close the lid.

   Note: You may use an elution volume of ≤150 µL for elution (page 16).
3. Incubate at room temperature for 1 minute.
4. Centrifuge the column at maximum speed for 1 minute. The Recovery Tube contains purified viral nucleic acids. Remove and discard the spin column.
5. Store the purified RNA/DNA at –80°C or use the RNA/DNA for the desired downstream application.

Analyzing Viral RNA/DNA

Since the amount of viral RNA/DNA present in cell-free body fluids is low and there is considerable amount of Carrier RNA in the purified viral RNA/DNA sample, we do not recommend using UV absorbance at 260 nm or Quant-iT™ Assay Kits to determine the viral nucleic acid yields.

To determine viral nucleic acid yield, use qRT-PCR or RT-PCR for RNA virus, and qPCR and PCR for DNA virus using appropriate viral-specific probes.

To analyze viral nucleic acid size, use agarose gel electrophoresis followed by hybridization using viral specific labeled probes and autoradiography.

Example of Expected Results for Mini Kit

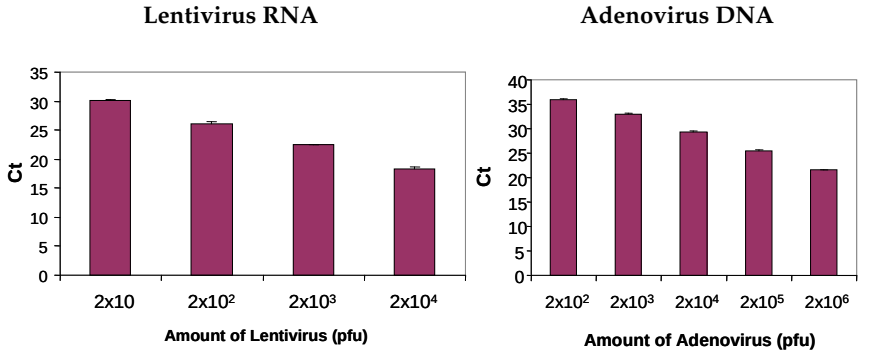
Results

Examples of results obtained after purification of viral RNA/DNA using the PureLink® Viral RNA/DNA Mini Kit are shown in the following figure.

Serum samples (200 µL) were spiked with lentivirus RNA (left panel) or adenovirus DNA (right panel) at the indicated pfu. Viral RNA/DNA was purified using the PureLink® Viral RNA/DNA Mini Kit as described in this manual. Elution was performed with 50 µL RNase-free water.

For lentivirus RNA sample, 5 µL of the purified RNA was used to perform qRT-PCR using the **SuperScript™ III Platinum® One-Step qRT-PCR Kit w/ROX** with TaqMan® primers in an ABI 7700 instrument. For adenovirus DNA sample, 5 µL of purified DNA was used to perform qPCR using the **Platinum® Quantitative PCR SuperMix-UDG w/ROX** kit (page 31) with LUX™ primers in an ABI 7700 instrument.

Results: Consistently lower Ct values indicate an increase in sensitivity of detection for viral RNA and DNA using the PureLink® Viral RNA/DNA Mini Kit.



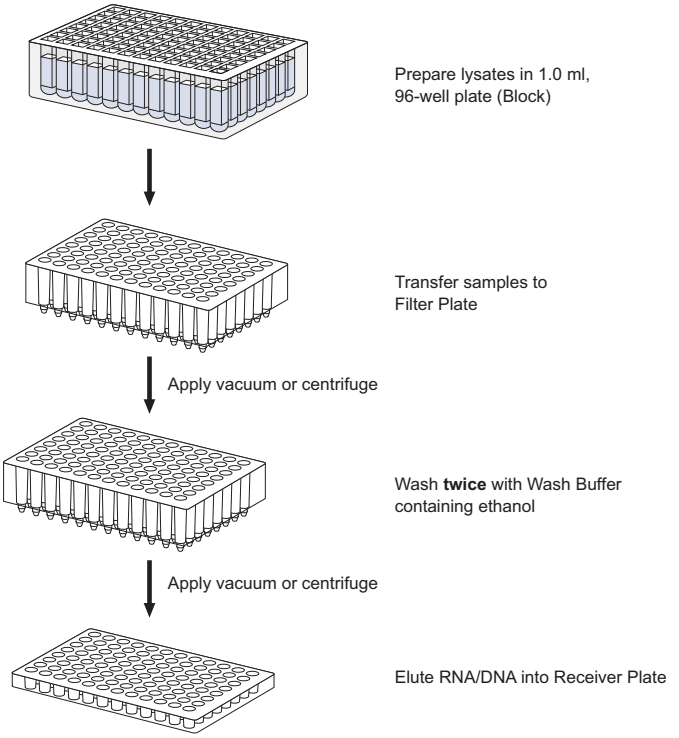
Purification Procedure for 96 Kit

Introduction

The viral nucleic acid purification procedure described here uses plate-based centrifugation or vacuum for high-throughput processing of samples.

Experimental Overview

The flow chart for purifying viral RNA/DNA using the PureLink® 96 Viral RNA/DNA Kit is shown in the following diagram.



Purification Procedure for 96 Kit, Continued

Follow the recommendations below to obtain the best results:

- Perform all centrifugation steps at room temperature
- Be sure to add ethanol to Wash Buffer (W5) before use (page 12)
- Perform the recommended wash steps to obtain high-quality RNA
- Always pipet water in the center of the silica matrix and perform a 1 minute incubation before elution

Important

- Handle all viruses in compliance with established institutional guidelines. Since safety requirements for use and handling of viruses may vary at individual institutions, we recommend consulting the health and safety guidelines and/or officers at your institution.
- Be sure to take the appropriate precautions (wear a laboratory coat, disposable gloves, and eye protection) when handling viral samples.
- The eluates collected during wash steps contain biohazardous waste. Dispose the eluate and collection tubes appropriately as biohazardous waste.

Elution Volume

The PureLink® 96 Viral RNA/DNA Kit elutes viral nucleic acid in 100–150 µL for downstream applications.

Note: Using larger elution volume decreases the viral nucleic acid concentration in the eluate.

Purification Procedure for 96 Kit, Continued

Automation Scripts

For automation scripts, contact Technical Support page 32.

Materials Needed

- Cell-free samples (such as plasma or serum samples)
- Appropriate amount of Lysis Buffer (L22) containing Carrier RNA, page 13
- Heat block for 96-well plate, water bath, or incubator set to 56ºC
- 96–100% ethanol
- Sterile, RNase-free PBS or 0.9% NaCl
- Vacuum manifold and a vacuum pump for 96-well plates (producing pressure of –12 to –15 in. Hg or –800 to –900 mbar) or automated liquid handling workstations

  or

- Centrifuge with buckets to hold 96-well plates and are 5 cm (2 inches) deep. Centrifuge speed must be ≥2250 × *g* and temperature should be 25°C

Components supplied with the kit

- Wash Buffer (W5)
- Sterile, RNase-free Water (E3)
- 96-well PureLink® Filter Plates
- 96-well Receiver Plates
- 96-well Deep-Well Plates
- Foil tape

Purification Procedure for 96 Kit, Continued

Preparing Lysate

The lysate preparation protocol is described below for **200 µL** starting material.

1. Take one Deep-Well Plate and add 25 µL Proteinase K per well for each sample.
2. Add 200 µL of cell-free sample (equilibrated to room temperature) to each well.

   Note: If sample is less than 200 µL, adjust the final volume of the sample to 200 µL using sterile PBS or 0.9% NaCl.
3. Add 200 µL Lysis Buffer (containing 5.6 µg Carrier RNA). Mix by pipetting up and down 3–5 times.
4. Seal the plate with the foil tape and incubate at 56ºC for 15 minutes.
5. Centrifuge the plate briefly to collect the liquid in the bottom of the well.
6. Add 250 µL 96–100% ethanol to each well to obtain a final ethanol concentration of 37% and mix by pipetting up and down 3–5 times.

   Note: If you are processing multiple samples, you may add ethanol to all wells and then mix.
7. Incubate the lysate with ethanol for 5 minutes at room temperature.
8. If you are using centrifugation for purification, follow the procedure on page 25. If you are using a vacuum manifold for purification, proceed to page 26.

Purification Procedure for 96 Kit, Continued

Purification Procedure Using Centrifugation

1. Transfer each lysate sample to a well of the 96-well Filter Plate. Cover any unused wells with foil tape.
2. Place the 96-well Filter Plate onto a new or used 96-well Deep-Well Plate and centrifuge at ≥2250 × *g* for 5 minutes.
3. Remove the flow-through and place the Filter Plate back on the Deep-Well Plate.
4. Add 500 µL Wash Buffer (W5) with ethanol into each well of the 96-well Filter Plate.
5. Centrifuge at maximum speed until all liquid passes through the Filter Plate, about 5 minutes.
6. Remove the flow-through and place the Filter Plate back on the Deep-Well Plate.
7. Repeat Steps 4–6.
8. Centrifuge the Filter Plate at ≥2250 × *g* for 5 minutes to dry the plate completely.
9. Add 100 µL RNase-free water (E3) to the center of the membrane in each well and incubate the plate for 1 minute at room temperature.
10. Place the Filter Plate onto a Receiver Plate (supplied in the kit). Centrifuge at ≥2250 × *g* for 5 minutes.
11. Use the RNA/DNA for the desired downstream application. To store the purified RNA/DNA, cover the wells with foil tape, and store at –80°C.

Purification Procedure for 96 Kit, Continued

Purification Procedure Using a Vacuum Manifold

Assemble the vacuum manifold as per the manufacturer's instructions.

1. Place a 96-well Filter Plate into the vacuum manifold designed to hold a 96-well plate.
2. Transfer all of the lysates from each well of the Deep-Well Plate to a fresh well in the Filter Plate. Cover unused wells with foil tape.
3. Apply vacuum for 2 minutes at room temperature and release vacuum.
4. Add 500 µL Wash Buffer (W5) with ethanol into each well of the 96-well Filter Plate.
5. Apply vacuum for 2 minutes at room temperature. Release vacuum.
6. Repeat Steps 4 and 5.
7. After releasing the vacuum, tap the Filter Plate with the filter side down on a stack of paper towels and pat dry the plate. Alternatively, you can centrifuge the Filter Plate at ≥2250 × *g* for 5 minutes to dry the plate completely.
8. Place the Filter Plate on the vacuum manifold and apply vacuum for 10 minutes at room temperature. Release vacuum.
9. Proceed to elution using vacuum manifold (Steps 10–12, below) or using centrifugation (Steps 9–10, page 25).
10. Place the Receiver Plate included in the kit in the vacuum manifold in place of the waste collection tray and place the Filter Plate on top of the Receiver Plate.
11. Add 150 µL of Sterile, RNase-free water (E3) to the center of the membrane in each well of the Filter Plate and incubate the plate for 1 minute at room temperature.
12. Apply vacuum for 2 minutes at room temperature. Release vacuum. The RNA/DNA is eluted into the Receiver Plate in a volume of 120–130 µL.
13. Use the RNA/DNA for the desired downstream application. To store the purified RNA/DNA, cover the wells with foil tape, and store at –80°C.

Purification Procedure for 96 Kit, Continued

Analyzing Viral RNA/DNA

Since the amount of viral RNA/DNA present in cell-free body fluids is low we recommend that you do not use UV absorbance to determine yield. Use qRT-PCR or RT-PCR for RNA virus, and qPCR and PCR for DNA virus using appropriate viral-specific probes to determine yield or presence of viral nucleic acid.

To analyze viral nucleic acid size, use agarose gel electrophoresis followed by hybridization using viral specific labeled probes and autoradiography.

Example of Expected Results for 96 Kit

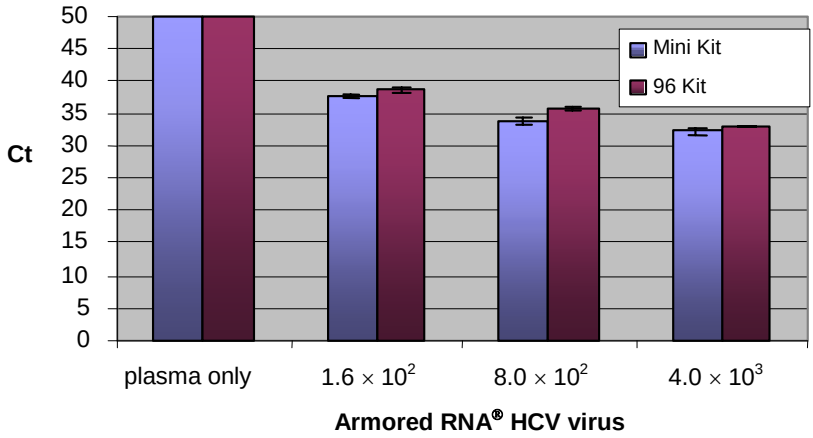
Results

Examples of results obtained after purification of viral RNA/DNA using the PureLink® 96 Viral RNA/DNA Kit are shown in the following figure.

Serum or plasmid samples (200 µL) were spiked with Armored RNA® Hepatitis C Virus (HCV) virus at the indicated pfu. Viral RNA was purified using the PureLink® 96 Viral RNA/DNA Kit or the PureLink® RNA/DNA Mini Kit as described in this manual.

qRT-PCR was performed on the resulting samples using the SuperScript™ III One-Step qRT-PCR Kit w/Platinum® *Taq* with TaqMan® primers in an ABI 7700 instrument.

Results: Consistently lower Ct values indicate an increase in sensitivity of detection for viral RNA and comparable performance using either PureLink® kit.



Troubleshooting

Problem	Cause	Solution
Low nucleic acid yield	Incomplete lysis or the column or matrix is clogged	Be sure to use the appropriate volumes of reagents during lysate preparation. If you are processing >200 µL sample for the Mini Kit, adjust the reagent volumes accordingly to obtain complete lysis. Do not use more than 200 µL sample when using the 96 Kit. If cryoprecipitates are visible in frozen viral samples, remove the cryoprecipitates by centrifugation (page 12) to avoid clogging of the column or matrix. Precipitates in buffers may affect lysis. Dissolve precipitates by warming the buffers at 25°C to 37°C.
	Poor quality of sample material	Avoid repeated freezing and thawing of samples. Use fresh samples and process immediately after collection or use samples thawed only once for best results. Check the quality of the RNA in the original samples using qRT-PCR or RT-PCR.
	Lysis Buffer without Carrier RNA used or Carrier RNA inactivated	To prepare lysates from cell-free samples, use Lysis Buffer (L22) with Carrier RNA (page 13). Once the Carrier RNA is reconstituted in water, aliquot the Carrier RNA and store at –20°C. Do not perform multiple freeze-thaw cycles.
	Incorrect binding conditions	For efficient binding of viral nucleic acids, always **add** ethanol to the lysate to a final concentration of 37% prior to loading the lysate onto the column or matrix.
	Ethanol not added to Wash Buffer (W5)	Be sure to add 96–100% ethanol to Wash Buffer (W5) as described on page 12. Do not use denatured 95% ethanol.
	Incorrect elution conditions	Add water to the center of the silica matrix and perform incubation for 1 minute with water before eluting.

Troubleshooting, Continued

Problem	Cause	Solution
Low nucleic acid yield, continued	RNA quantitation performed using UV absorbance	Since viral nucleic acids are present in low amounts in cell-free samples, **do not** use UV absorbance for quantitation. Analyze viral nucleic acids using qRT-PCR, RT-PCR, qPCR, or PCR.
RNA degraded	RNA contaminated with RNase	Follow the guidelines on page 11 to prevent RNase contamination.
	Poor quality of samples	Always use fresh samples or samples frozen at –80°C. For lysis, process the sample quickly to avoid degradation.
Poor performance of nucleic acids in downstream enzymatic reactions	Presence of ethanol or use of denatured 95% ethanol in purified nucleic acids	Traces of ethanol from the Wash Buffer (W5) can inhibit downstream enzymatic reactions. To remove Wash Buffer (W5), discard Wash Buffer (W5) flow through. Always use a new Wash Tube or Receiver Plate and completely dry the column or membrane. Use only 96–100% ethanol. Do not use denatured 95% ethanol.
	Assay may be sensitive to Carrier RNA concentration	You may need to optimize the amount of Carrier RNA that is required for optimal purification and is suitable for your downstream applications.
	Reagents for enzymatic reactions inactive	Ensure that the enzymes and reagents used for performing downstream applications have not expired or inactivated. Repeat the reaction with fresh enzyme and reagents.
	Viral nucleic acid eluate too dilute	Optimize the amount of viral nucleic acid eluate required for your specific application and perform elution using the desired kit and elution volume (10–150 µL).
Carrier RNA not enough to process samples	Incorrect Carrier RNA amount used per sample	We recommend using a maximum of 5.6 µg Carrier RNA per sample when processing ≤500 µL sample volume. **Do not** increase the Carrier RNA amount when processing 500 µL sample volume using the Mini Kit.

Appendix

Accessory Products

Additional Products

The following products are also available from Life Technologies. For details on these products, visit **www.lifetechnologies.com** or contact Technical Support (page 32).

Product	Quantity	Catalog No.
Wash Tubes (2.0 mL)	100	12282-100
RNase *AWAY*®	250 mL	10328-011
PureLink® Foil Tape	50 pieces	12261-012
Viral Lysis Buffer (L22)	500 mL	12282-500
UltraPure™ DEPC-treated Water	1 L	750023
UltraPure™ DNase/RNase-Free Distilled Water	500 mL	10977-015
Yeast tRNA	25 mg	15401-011
Phosphate Buffered Saline (PBS), 1X	500 mL	10010-023
SuperScript™ III One-Step RT-PCR System with Platinum® *Taq* DNA Polymerase	100 reactions	12574-026
RNA UltraSense™ One-Step Quantitative RT-PCR System	100 reactions	11732-927
Platinum® Quantitative PCR SuperMix-UDG w/ROX	100 reactions	11743-100

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Purchaser Notification

Robotic Scripts Disclaimer

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