invitrogen™

PureLink™ Pro 96 Viral RNA/DNA Kit

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

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MAN0001672

User Manual

Contents

Experienced Users Procedure

Introduction

This quick reference sheet is included for experienced users of the PureLink™ Pro 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** of starting material. 1. Add 25 µl Proteinase K to each well. 2. Add 200 µl of cell-free sample to the well. **Note:** If processing <200 µl sample, adjust the 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 PureLink™ 96 Well Viral Filter Plate. Cover unused wells with foil tape. 2. Place the Viral Filter Plate onto a 96-well, Deep-Well Block and centrifuge at ≥2,100 × g for 1–2 minutes. 3. Remove the flow-through and place the Viral Filter Plate back on the Deep-Well Block. 4. Add 500 µl Wash Buffer II with ethanol. 5. Centrifuge 5 minutes at ≥2,100 × g until liquid passes through the Viral Filter Plate. 6. Remove the flow-through and place the Viral Filter Plate back on the Deep-Well Block. 7. Repeat Steps 4–6 above. 8. Centrifuge the Viral Filter Plate at ≥2,100 × g for 10 minutes. 9. Add 100 µl RNase-free water and incubate the plate for 1 minute at room temperature. 10. Place the Viral Filter Plate onto a clean Deep-Well Block. Centrifuge at ≥2,100 × g for 1–2 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.

Continued on next page

Experienced Users Procedure, Continued

Step	Action
Purification Procedure with Vacuum	Prepare lysate as described on previous page. Perform all steps at 25°C. 1. Place a PureLink™ 96 Well Viral RNA/DNA Filter Plate on top of the manifold. 2. Transfer lysates from Preparing Lysates, Step 6 to the Viral Filter Plate. Cover unused wells with foil tape. 3. Apply vacuum for 2 minutes. Release the vacuum. 4. Add 500 µl Wash Buffer II with ethanol. 5. Apply vacuum 2 minutes. Release the vacuum. 6. Repeat Steps 4 and 5 above. 7. Tap the Viral Filter Plate on a stack of paper towels and pat dry. 8. Return the Viral Filter Plate to the vacuum manifold and apply vacuum for 10 minutes. Release the vacuum. 9. Replace the waste collection tray with a clean Deep-Well Block and place the Viral Filter Plate on top of the Deep-Well Block. 10. Add 150 µl RNase-free water and incubate the plate for 1 minute at room temperature. 11. Apply vacuum for 2 minutes. Release vacuum. 12. The RNA/DNA is eluted into the Deep-Well Block in a volume of 110–130 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™ Pro 96 Viral RNA/DNA Kit is shipped at room temperature.

Upon receipt, store all kit components **except Carrier RNA** at room temperature.

S**tore 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.

Contents

The components and amounts included in the PureLink™ Pro 96 Viral RNA/DNA Kit is listed below.

Sufficient reagents are in the kit to perform 384 (4 × 96) reactions.

Component	Amount
PureLink™ Pro 96 Viral Lysis Buffer (L22)	100 ml
PureLink™ Pro 96 Wash Buffer II (5X)	2 × 87.5 ml
Proteinase K (20 mg/ml) in storage buffer (proprietary)	10 ml
Carrier RNA (lyophilized)	2.2 mg
PureLink™ Pro 96 RNase-free Water	75 ml
PureLink™ 96 Well Viral RNA/DNA Filter Plate	4
96 Deep-Well Block	2 × 6/bag
Foil Tape	3 × 4/bag

Accessory Products

Additional Products

The following products are also available from Invitrogen. For details on these products, visit www.invitrogen.com or contact Technical Support (page 17).

Product	Quantity	Catalog no.
RNase *AWAY*®	250 ml	10328-011
RNase*ZAP*®	250 ml	AM9780
PureLink™ Foil Tape	50 pieces	12261-012
Viral Lysis Buffer (L22)	500 ml	12282-500
PureLink™ 96 RNA Wash Buffer II (5X)	2 x 1 L	12173-033
EveryPrep™ Universal Vacuum Manifold	1 manifold	K211101
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

Introduction

About the Kit

Introduction

The PureLink™ Pro 96 Viral RNA/DNA Kit provides 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™ Pro 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

The kit allows 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 56ºC. 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 PureLink™ 96 Well Viral RNA/DNA Filter Plate. 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.

Continued on next page

About the Kit, Continued

Advantages

The PureLink™ Pro 96 Viral RNA/DNA Kit provides the following advantages:

- Highest sensitivity for samples with very low viral titer
- High-throughput processing of samples
- Automation using Tecan or Beckman robotic systems
- 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™ Pro 96 Viral RNA/DNA Kit is yeast tRNA (page viii). 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 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.

Continued on next page

About the Kit, 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

Kit Specifications

Dimensions:	Standard SBS (Society for Biomolecular Screening) footprint
Starting Material:	Up to 200 µl cell-free sample
Binding Capacity:	At least 40 µg nucleic acid
Filter Reservoir Capacity:	1.0 ml
Deep-Well Block Capacity:	1.09 ml
Centrifuge Compatibility:	Capable of centrifuging at ≥2,100 × g Bucket depth 7 cm
Elution Volume:	100–150 µl

Methods

Before Starting

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.

The PureLink™ Viral RNA/DNA Kit buffers contain guanidine isothiocyanate. Always wear a laboratory coat, disposable gloves, and eye protection when handling buffers.

Do not add bleach or acidic solutions directly to solutions containing guanidine isothiocyanate or sample preparation waste as it forms reactive compounds and toxic gases when mixed with bleach or acids.

Important

If there is any precipitate present in the buffers, warm the buffer up to 25–37°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
- Always use proper microbiological aseptic techniques when working with RNA
- Use RNase *AWAY*® Reagent (page viii) to remove RNase contamination from surfaces (including from non-disposable equipment like homogenizers)

Continued on next page

Before Starting, Continued

Starting Material

The PureLink™ Pro 96 Viral RNA/DNA Kit is 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 (e.g. plasma or serum) and proceed immediately to purification. If desired, store the sample at 4ºC for short-term storage (up to 4 hours) or freeze the sample at –20ºC or –80ºC for long-term storage.
- Do not freeze-thaw plasma or serum samples more than once.
- Remove any visible cryoprecipitates from samples by centrifugation at ~7,000 × 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™ Pro 96 Viral RNA/DNA Kit can process sample volumes of up to 200 µl without preparation of any additional buffers or carry over of any impurities.

Preparing Wash Buffer

Add 350 ml of 96–100% ethanol to 87.5 ml PureLink™ Pro 96 Wash Buffer II included in the kit.

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

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Preparing Carrier RNA

The recommended purification protocol uses 5.6 µg Carrier RNA per sample (for up to 200 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 with the kit) to obtain a 1 µg/µl Carrier RNA stock solution.
2. Mix thoroughly and divide 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 formula below:

   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).

Continued on next page

Before Starting, Continued

Instrument Compatibility for 96-Well Plates

The PureLink™ 96 Well Viral RNA/DNA Filter Plates are compatible with the following instruments:

- **Vacuum Manifold:** The manifold must accommodate the PureLink™ 96 Well Plate (half-skirted filter plate) and be capable of collecting the filtrate (e.g. EveryPrep™ Universal Vacuum Manifold from Invitrogen).
- **Centrifuge:** The centrifuge must be capable of centrifuging 96-well plates at ≥2,100 × g, and accommodate a 7.0 cm microtiter plate stack.
- **Automated Liquid Handling Workstation:** The workstation must be equipped with a vacuum manifold and a vacuum source, and accommodate the PureLink™ 96 Well Plate (half-skirted filter plate).

Calibrating Vacuum for Use with 96-Well Plates

Use 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™ 96 Well Viral RNA/DNA 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.

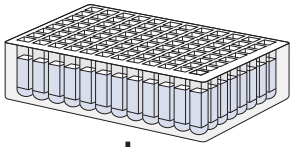
   Note: During purification the vacuum pressure may exceed the recommended value.

Experimental Overview

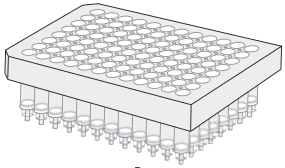
The viral nucleic acid purification procedure described below uses plate-based centrifugation or vacuum for high-throughput processing of samples.

Workflow

The flow chart for purifying viral RNA/DNA using the PureLink™ Pro 96 Viral RNA/DNA Kit is shown below.

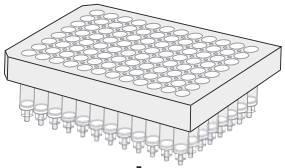


Prepare lysates with Proteinase K and Viral Lysis Buffer (L22) in 96 Deep-Well Block



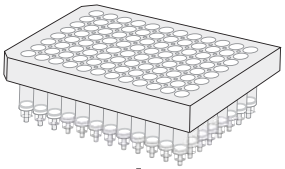
Transfer samples to Viral RNA/DNA Filter Plate

Apply vacuum or centrifuge



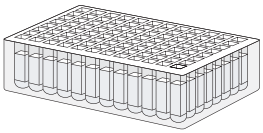
Wash **twice** with Wash Buffer II containing ethanol

Apply vacuum or centrifuge



Elute RNA/DNA with RNase-free Water

Apply vacuum or centrifuge



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General Guidelines

Follow the recommendations below to obtain the best results:

- Perform all steps at room temperature
- Be sure to add ethanol to Wash Buffer II before use (page 5)
- Perform the recommended wash steps to obtain high-quality RNA
- 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™ Pro 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.

Automation Scripts

For automation scripts, contact Technical Support page 17.

Continued on next page

Preparing Lysates

Materials Needed

- Cell-free samples (such as plasma or serum samples)
- Appropriate amount of Lysis Buffer (L22) containing Carrier RNA (see page 6)
- Heat block for 96-well plate, water bath, or incubator set to 56ºC
- 96–100% ethanol
- 96-well Deep-Well Blocks (supplied with kit)
- Sterile, RNase-free PBS or 0.9% NaCl
- Centrifuge capable of ≥2,100 × g with buckets that can accommodate 96-well plates that have a plate height clearance of 7.0 cm

Preparing Lysate

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

1. Add 25 µl Proteinase K into each well of a Deep-Well Block that contain samples.
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. Proceed to **Purification Using Centrifugation** (page 11), **Purification Using a Vacuum Manifold** (page 12), or **Purification Using the EveryPrep™ Universal Vacuum Manifold** (page 17).

Purification Using Centrifugation

Materials Needed

- Viral lysate from cell-free samples (see previous page)
- Centrifuge capable of ≥2,100 × g with buckets that can accommodate 96-well plates that have a plate height clearance of 7.0 cm
- PureLink™ Pro 96 Wash Buffer II with ethanol (page 5)
- PureLink™ Pro 96 RNase-free Water (supplied with kit)
- PureLink™ 96 Well Viral RNA/DNA Filter Plate (supplied with kit)
- 96-well Deep-Well Blocks (supplied with kit)
- Foil tape (supplied with kit)

Purification Procedure Using Centrifugation

Perform all steps at room temperature:

1. Transfer each lysate sample to a well of the PureLink™ 96 Well Viral RNA/DNA Filter Plate. Cover any unused wells with foil tape.
2. Place the Viral Filter Plate onto a new or used 96-well Deep-Well Block and centrifuge at ≥2,100 × g for 1–2 minutes.
3. Discard the flow-through and place the Viral Filter Plate back on the Deep-Well Block.
4. Add 500 µl Wash Buffer II (with ethanol) into each well of the Viral Filter Plate.
5. Centrifuge at ≥2,100 × g for 1–2 minutes, until all liquid passes through the Viral Filter Plate.
6. Discard the flow-through and place the Viral Filter Plate back on the Deep-Well Block.
7. Repeat Steps 4–6 above.
8. Centrifuge the Viral Filter Plate at ≥2,100 × g for 10 minutes to dry the plate completely.
9. Add 100 µl RNase-free water to the center of each well and incubate the plate for 1 minute at room temperature.
10. Place the Viral Filter Plate onto a new Deep-Well Block (supplied in the kit). Centrifuge at ≥2,100 × g for 1–2 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 Using a Vacuum Manifold

Materials Needed

- Viral lysate from cell-free samples (see previous page)
- Vacuum manifold and a vacuum pump (producing pressure of –12 to –15 in. Hg) or automated liquid handling workstation
- PureLink™ Pro 96 Wash Buffer II with ethanol (page 5)
- PureLink™ Pro 96 RNase-free Water (supplied with kit)
- PureLink™ 96 Well Viral RNA/DNA Filter Plate (supplied with kit)
- 96-well Deep-Well Blocks (supplied with kit)
- Foil tape (supplied with kit)

Binding and Washing RNA/DNA

Assemble the vacuum manifold according to the manufacturer's instructions. For a protocol using the EveryPrep™ Universal Vacuum Manifold, see page 17.

1. Place a PureLink™ 96 Well Viral RNA/DNA Filter Plate on top of the vacuum manifold.
2. Transfer the lysates from the Deep-Well Block to the Viral Filter Plate. Cover unused wells with foil tape.
3. Apply vacuum for 2 minutes. Release vacuum.
4. Add 500 µl Wash Buffer II (with ethanol) into each well of the Viral Filter Plate.
5. Apply vacuum for 2 minutes. Release vacuum.
6. Repeat Steps 4 and 5 above.
7. Place the Viral Filter Plate on the vacuum manifold and apply vacuum for 10 minutes. Release vacuum.

   Alternatively, centrifuge the Viral Filter Plate at ≥2,100 × g for 10 minutes to dry the plate completely.
8. Place the Viral Filter Plate with the filter side down on a stack of paper towels, and pat firmly to blot any residual liquid.
9. Proceed to elution using vacuum manifold (Steps 10–12, next page), or using centrifugation (Steps 9–10, previous page).

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Purification Using a Vacuum Manifold,
Continued

Eluting RNA/DNA

10. Place a clean Deep-Well Block (included in the kit) in the vacuum manifold in place of the waste collection tray and place the Viral Filter Plate on top of the Deep-Well Block.
11. Add 150 µl of RNase-free water (included in the kit) to the center of the membrane in each well of the Viral Filter Plate and incubate for 1 minute at room temperature.
12. Apply vacuum for 2 minutes. Release vacuum. The RNA/DNA is eluted into the Deep-Well Block in a volume of 110–130 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.

Example of Expected Results

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.

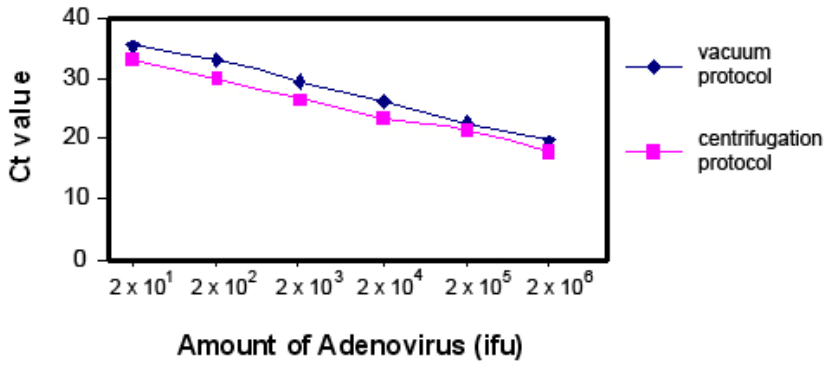
Results

Examples of results obtained after purification of viral RNA/DNA using the PureLink™ Pro 96 Viral RNA/DNA Kit are shown below.

Plasma samples (200 µl) were spiked with Adenovirus at the indicated ifu (infectious unit). Viral DNA were purified using the PureLink™ 96 Viral RNA/DNA Kit as described in this manual.

qPCR was performed on the resulting samples using the Platinum® quantitative PCR Supermix-UDG Kit with TaqMan® primers in an ABI 7900HT instrument.

Consistent recovery of viral DNA is reflected by linear performance of the PureLink™ Pro 96 Viral RNA/DNA Kit when analyzed by qPCR.



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. Do not use more than 200 µl sample. If cryoprecipitates are visible in frozen viral samples, remove the cryoprecipitates by centrifugation (page 5) to avoid clogging of the column or matrix. Precipitates in buffers may affect lysis. Dissolve precipitates by warming the buffers at 25–37°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 6). 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 II	Be sure to add 96–100% ethanol to Wash Buffer II as described on page 5. 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.

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Troubleshooting, Continued

Problem	Cause	Solution
Low nucleic acid yield, continued	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 4 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 II can inhibit downstream enzymatic reactions. To remove residual buffer, discard Wash Buffer II flow through. Completely dry the Viral Filter Plate, and always use a new Deep-Well Block for elution. 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 up to 200 µl sample volume.

Appendix

Purification Using the EveryPrep™ Universal Vacuum Manifold

Introduction

Instructions are provided below to purify DNA using the EveryPrep™ Universal Vacuum Manifold (see page viii). Refer to the manual for the EveryPrep™ Universal Vacuum Manifold for detailed instructions on operation with the 96 Well Top Plate. All steps are performed at room temperature.

Materials Needed

- Viral lysate from cell-free samples (see previous page)
- Vacuum manifold and vacuum pump (producing pressure of 12–15 in. Hg)
- PureLink™ Pro 96 Wash Buffer II with ethanol (page 5)
- PureLink™ Pro 96 RNase-free Water (supplied with kit)
- PureLink™ 96 Well Viral RNA/DNA Filter Plate (supplied with kit)
- 96-well Deep-Well Blocks (supplied with kit)
- Foil tape (supplied with kit)

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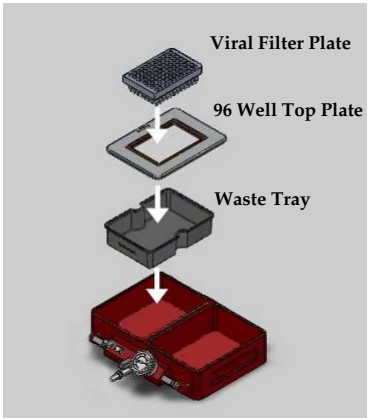
Purification Using the EveryPrep™ Universal Vacuum Manifold, Continued

EveryPrep™ Universal Vacuum Manifold Assembly

1. Assemble the EveryPrep™ Universal Vacuum Manifold: Place the Waste Tray in the Binding Chamber, cover the top with the 96 Well Top Plate, and place the PureLink™ Viral RNA/DNA Filter Plate over the Top Plate.



2. Proceed to **Binding RNA/DNA**, below.

Binding and Washing RNA/DNA

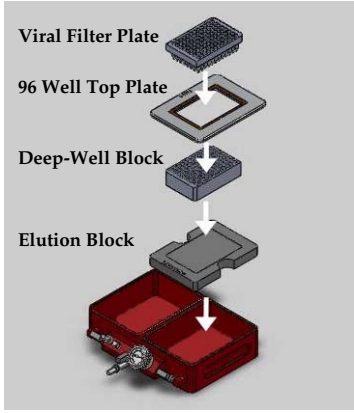
1. Transfer the lysates from the Deep-Well Block to the Viral Filter Plate. Cover unused wells with foil tape.
2. Apply vacuum for 2 minutes. Release vacuum.
3. Add 500 µl Wash Buffer II (with ethanol) into each well of the Viral Filter Plate.
4. Apply vacuum for 2 minutes. Release vacuum.
5. Repeat Steps 4 and 5 above.
6. Apply vacuum for an additional 10 minutes. Release vacuum.
7. Place the Viral Filter Plate with the filter side down on a stack of paper towels, and pat firmly to blot any residual liquid.
8. Proceed to **Eluting RNA/DNA**, next page.

Continued on next page

Purification Using the EveryPrep™ Universal Vacuum Manifold, Continued

Eluting RNA/DNA

1. Prepare the EveryPrep™ Universal Vacuum Manifold for elution: Place the Elution Block and a clean Deep-Well Block in the Elution Chamber, cover the top with the 96 Well Top Plate, and place the PureLink™ Viral RNA/DNA Filter Plate over the Top Plate.



2. Add 150 µl of RNase-free water (included in the kit) to the center of the membrane in each well of the Viral Filter Plate and incubate for 1 minute at room temperature.
3. Apply vacuum for 2 minutes. Release vacuum. The RNA/DNA is eluted into the Deep-Well Block in a volume of 110–130 µl.
4. 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.

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Purchaser Notification, Continued

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