invitrogen™
by life technologies™

# iPrep™ PureLink™ Virus Kit

## For purification of viral nucleic acids from cell-free samples using the iPrep™ Purification Instrument

**Catalog no. IS10008**

**Rev. date: 15 August 2010**
Manual part no. 100004281

MAN0000409

**User Manual**

# Contents

# Experienced Users Procedure

## Introduction

This quick reference sheet is included for experienced users of the iPrep™ PureLink™ Virus Kit. For more details, refer to this manual.

| Step | Procedure |
|---|---|
| Purification Protocol | 1. Mix fresh cell-free samples or thaw frozen cell-free samples or lyse bacteria using lysozyme for bacterial gDNA isolation (page 9).<br>**Note:** The need for lysozyme digestion step is dependent on the type of bacteria in use as certain Gram negative bacteria (e.g., *E. coli*) do not need the lysozyme digestion step. If lysozyme digestion step is not performed, use 200 µL or 400 µL bacterial samples.<br>2. Open the iPrep™ Card Slot and insert the **iPrep™ Card: Viral DNA/RNA** in the slot (arrow on the card is at the top and card label is facing your left side).<br>3. Turn **ON** the iPrep™ Instrument using the power switch on the left side of the instrument.<br>The digital display shows the version for the iPrep™ which changes in few seconds to display the **Main** menu.<br>4. Press **Start** to run a protocol.<br>5. Open the iPrep™ instrument door and remove iPrep™ Racks to set up the platform.<br>6. Remove the iPrep™ PureLink™ Virus Cartridges from the box. To collect any solution from the foil, tap the cartridge to deposit the solution at the bottom of the tube.<br>7. Insert one iPrep™ Sample Processing Tube (2 mL) in the **heated tube position** of cartridge **(position 11)** for each iPrep™ PureLink™ Virus Cartridge that is used.<br>8. Load the cartridges on the iPrep™ Cartridge Rack and insert the loaded rack on to the iPrep™ Platform. |

*Continued on next page*

# Experienced Users Procedure, Continued

| Step | Procedure |
|---|---|
| Purification Protocol, Continued | 9. Load the iPrep™ Tip and Tube Rack as follows:<br>• Load the first row (labeled as **E**) with 1–13 elution tubes **without caps**<br>• Load the second row (labeled as **T1**) with iPrep™ Small Tips (blue tips, page 19) in iPrep™ Tip Holders for eluting samples in 20 µL elution volume<br>• Load the third row (labeled as **T2**) with iPrep™ Tips in iPrep™ Tip Holders<br>• Load the fourth row (labeled as **S**) with iPrep™ Sample and Elution Tubes **containing samples**<br>10. Read the sample and elution tube barcodes, if needed.<br>11. Insert the iPrep™ Tip and Tube Rack on to the iPrep™ Platform.<br>12. Close the iPrep™ instrument door. Press **Enter** (↵)to continue.<br>13. When prompted, select the appropriate lysis mode, sample volume, and elution volume.<br>14. Press **Start**. The automated purification protocol begins and various steps of the protocol including the approximate time remaining are displayed on the digital display.<br>15. At the end of the run, the instrument beeps briefly and the digital display shows **Protocol Finished** for 10 seconds. The Main menu appears after 10 seconds.<br>16. Open the instrument door.<br>17. Remove and cap the elution tubes containing the purified nucleic acid. Store the purified RNA/DNA at –80°C.<br>18. Discard the used cartridges, tips, and tubes into biohazard waste. **Do not** reuse the cartridges.<br>19. To purify more samples using the same iPrep™ Card, load the racks with new cartridges, tips, tubes, and samples, and start the protocol as described.<br>20. If you are not using the instrument, close the instrument door and turn the power switch to **OFF**.<br>21. Remove the iPrep™ Card and store card in the box. |

# Kit Contents and Storage

## Shipping and Storage

The iPrep™ PureLink™ Virus Kit is shipped at room temperature.

Upon receipt, store the iPrep™ PureLink™ Virus Kit at room temperature. See below for kit contents.

All components are guaranteed stable for 6 months when stored properly.

## Kit Contents

The components supplied in the iPrep™ PureLink™ Virus Kit are listed below.

Sufficient reagents are supplied to perform 52 purifications.

| Reagents | Amount |
|---|---|
| iPrep™ PureLink™ Virus Cartridge Kit | 4 cartridge racks |
| iPrep™ Sample and Elution Tubes | 2 bags with 52 tubes |
| iPrep™ Sample Processing Tubes | 1 bag with 52 tubes |
| iPrep™ Tips and Tip Holders | 1 bag with 52 tips and holders |

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# Kit Contents and Storage, Continued

## iPrep™ PureLink™ Virus Cartridge Kit Contents

Each iPrep™ PureLink™ Virus Cartridge has 12 positions with 10 sealed wells and two heating positions (position 12 with an empty well and position 11 to add an empty or reagent filled tube).

Positions 1–10 contain wells filled with reagents for this protocol.

The components supplied in each well of the iPrep™ PureLink™ Virus Cartridge Kit are listed below.

**Store the iPrep™ PureLink™ Virus Cartridge Kit at room temperature and do not freeze the cartridge kit.**

| Reagent | Well no. |
|---|---|
| Viral Lysis Buffer | 1 |
| Proteinase K (20 mg/mL) in storage buffer (proprietary) | 2 |
| Elution Buffer (DEPC Water) | 3 |
| Empty | 4 |
| Wash Buffer 2 | 5 |
| Wash Buffer 2 | 6 |
| Wash Buffer 1 | 7 |
| Dynabeads® MyOne™ SILANE (4.8 mg/mL in Bead Storage Buffer) | 8 |
| Rinse Buffer | 9 |
| Isopropanol | 10 |

## Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

# Introduction

# Product Overview

## Introduction

The iPrep™ PureLink™ Virus Kit allows rapid and automated extraction of viral RNA/DNA as well as bacterial genomic DNA (gDNA) from fresh or frozen cell-free biological fluids (e.g., plasma, serum, urine) and cell culture supernatants.

Nucleic acid is purified from viral or bacterial cell-free samples using the Dynabeads® MyOne™ SILANE and iPrep™ Purification Instrument within 40 minutes without the use of centrifugation.

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

## iPrep™ Purification Instrument

The iPrep™ PureLink™ Virus Kit is designed for use with the iPrep™ Purification Instrument.

The iPrep™ Purification Instrument is a benchtop, automated nucleic acid purification instrument with integrated Magnetic and Syringe Unit capable of purifying nucleic acids from up to 13 samples (12 samples + 1 positive control) using magnetic bead-based technology. See page 3 for details on the iPrep™ Purification Instrument.

## System Overview

The iPrep™ PureLink™ Virus Kit combines the sensitivity and capacity of Dynabeads® MyOne™ SILANE with the speed and convenience of the iPrep™ Instrument to allow automated purification of high-quality DNA and RNA from up to 13 samples (12 samples + 1 positive control) within 40 minutes. The Dynabeads® MyOne™ SILANE are monodisperse magnetic beads (1 µm) with an optimized silica-like surface chemistry and a high specific surface area. Purification is achieved using magnetic bead-based procedure, and avoids the use centrifuges or vacuum manifolds.

The viral particles are lysed using Viral Lysis Buffer and proteins are digested with Proteinase K. The lysate is mixed with Dynabeads® MyOne™ SILANE for subsequent nucleic acid binding to the beads. The nucleic acid-bound magnetic beads are separated from the lysate using magnetic separation. The beads are thoroughly washed with Wash Buffers to remove contaminants. The RNA/DNA is eluted in Elution Buffer.

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# Product Product Overview, Continued

## Advantages

The iPrep™ PureLink™ Virus Kit provides the following advantages:

- Uses a magnetic bead-based technology to isolate viral RNA/DNA and bacterial gDNA without the need for centrifugation or vacuum manifolds
- Rapid and automated purification of nucleic acids within 40 minutes from a wide range of viral samples as well as Gram positive and Gram negative bacteria using the iPrep™ Instrument
- Pre-filled reagent cartridges provide easy set up and consistent results
- Minimal sample cross-contamination
- Purified nucleic acids demonstrate improved downstream performance in various applications

## System Specifications

| | |
|---|---|
| Starting Material: | 200 µL or 400 µL cell-free sample (see page 6) |
| Bead Size: | ~1 µm |
| Bead Amount per Reaction: | 2.4 mg |
| Number of Samples: | Up to 13 |
| Elution Volume: | 20 µL*, 50 µL, or 100 µL |

*To elute samples using 20 µL elution volume, you need to order iPrep™ Small Tips (page 19).

# iPrep™ Purification Instrument

## Introduction

The iPrep™ Purification Instrument is a benchtop, automated nucleic acid purification instrument with integrated Magnetic and Syringe Unit capable of purifying nucleic acids from up to 12 samples and one positive control. Each iPrep™ Instrument consists of the Magnetic and Syringe Unit, and a platform. A pre-programmed iPrep™ Protocol Card controls the purification parameters such as buffer volumes, mixing steps, and incubation time.

For more details on the iPrep™ Purification Instrument, see the manual supplied with the instrument.

## iPrep™ Reaction Cartridge

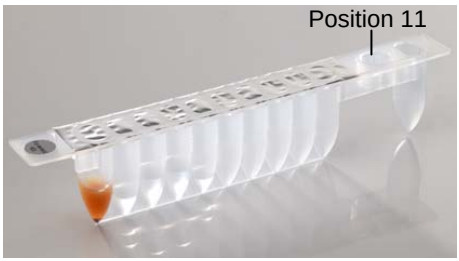
The iPrep™ Reaction Cartridges are supplied with iPrep™ Kits and are designed to fit onto the iPrep™ Cartridge Rack in only one orientation. Each cartridge is pre-filled with reagents required for the iPrep™ PureLink™ Virus protocol.

Each cartridge has 12 positions with 10 sealed wells and two heating positions (position 12 with an empty well and position 11 to add an empty or reagent filled tube). For the iPrep™ PureLink™ Virus Kit, positions 1–10 contain wells filled with reagents.

**Cartridge Specifications:**

| | |
|---|---|
| Material: | Polypropylene cartridge sealed with laminated aluminum foil |
| Max Volume: | 1,000 µL/well |
| Dimension: | 5.9 inches (l) × 1.2 inches (w) × 0.7 inches (d) |

**Note:** The image below shows an example of an iPrep™ Reagent Cartridge and is not an image of an iPrep™ Viral Cartridge.

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# iPrep™ Purification Instrument, Continued

## iPrep™ Tips and Tip Holders

The iPrep™ Tips and Tip Holders are included with iPrep™ Kits and are placed on the iPrep™ Tip and Tube Rack as described on page 11. While assembling tips on the rack, insert the iPrep™ Tips into the iPrep™ Tip Holders using gloved hands. Always use tips with the holders to prevent any contamination.

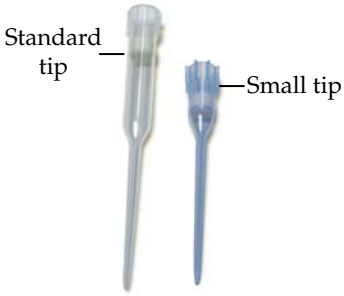
iPrep™ Small Tips are available separately from Invitrogen (page 19) and are used for lower elution volumes.

**Tip Specifications:**

| | |
|---|---|
| Tip Material: | Polypropylene with filter barriers |
| Tip Holder Material: | Polypropylene |
| Volume: | 5–1,000 µL (standard tips) |
| | 2–200 µL (small tips) |
| Standard Tip Dimensions: | 3.9 inches (l) × 0.43 inches (d) |
| Small Tip Dimensions: | 2.9 inches (l) × 0.43 inches (d) |

**iPrep™ Tip Holder**

**iPrep™ Tip**

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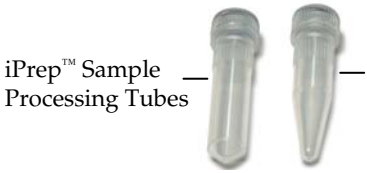
# iPrep™ Purification Instrument, Continued

## iPrep™ Tubes

Two sets of iPrep™ Tubes are required for the purification protocol. The iPrep™ Sample and Elution Tubes and iPrep™ Sample Processing tubes are included with each iPrep™ Viral Kit and placed on the iPrep™ Tip and Tube Rack as described on page 11.

**Tube Specifications:**

| | |
|---|---|
| Material: | Polypropylene |
| Capacity: | 1.5 mL (iPrep™ Sample and Elution Tubes)<br>2.0 mL (iPrep™ Sample Processing Tubes |
| Style: | Tubes with caps |
| Dimensions: | 1.7 inches (l) × 0.4 inches (d) |




## iPrep™ Card: Virus

To isolate viral RNA/DNA and bacterial gDNA using the iPrep™ PureLink™ Virus Kit with the iPrep™ Purification Instrument, you need to purchase the iPrep™ Card: Viral DNA/RNA (page 19).

The iPrep™ Card: Viral DNA/RNA is pre-programmed with the purification protocol for cell-free samples that directs the volume of reagents used and incubation time.

Always store the card in the box, protected from light.

To avoid damaging the card:

- Do not drop or bend the card
- Do not wipe or clean the card using volatile chemicals such as alcohol or equivalent
- Do not expose the card to water

## iPrep™ Platform

The platform on the iPrep™ Instrument allows the placement of iPrep™ Tip and Tube Rack, and iPrep™ Cartridge Rack that are filled with plastic disposables and reagent cartridges required for the purification protocol.

Set up the platform as shown in the figure on page 11 for the iPrep™ PureLink™ Virus Kit.

# Methods

# General Information

### User Supplied Materials

In addition to the reagents supplied with the kit, you also need the following materials and instrumentation:

- iPrep™ Purification Instrument (page 19)
- iPrep™ Card: Viral DNA/RNA (page 19)
- Viral or bacterial samples (see below)

### Cell-free Samples

The iPrep™ PureLink™ Viral Kit is designed to isolate viral and bacterial nucleic acids from cell-free biological fluids such as plasma, serum, and urine as well as cell culture supernatants using fresh or frozen samples.

To obtain high yield of 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 10). If desired, you can 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 the plasma or serum sample 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 iPrep™ PureLink™ Viral Protocol is designed to purify nucleic acids from **200 µL or 400 µL** cell-free samples.

**Do not** use less than 200 µL sample volume as using less sample volume results in excessive bubble formation during the purification protocol thereby lowering the yield.

*Continued on next page*

## Safety Information

Follow the safety guidelines below when using the iPrep™ PureLink™ Virus Kit.

- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles when handling samples.
- Dispose of viral and bacterial cell-free samples as biohazardous waste.


Follow the recommendations below to obtain the best results:

- Use disposable, individually wrapped, sterile plastic ware
- Use only sterile, new pipette tips (aerosol-barrier pipette 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 19) to remove RNase contamination from surfaces
- **Do not** freeze the beads as this irreparably damages them. Store the beads at room temperature.
- When using beads from the Reaction Cartridges, collect any solution from the foil by tapping the cartridge to deposit the solution at the bottom of the tube. **Do not** allow the beads to dry out as this renders them non-functional.
- Discard Reaction Cartridges, iPrep™ Tips, and iPrep™ Tip Holders after use. **Do not reuse.**

# Isolating RNA/DNA from Cell-free Samples

## Introduction

Instructions to isolate viral RNA/DNA and bacterial genomic DNA from cell-free samples using the iPrep™ PureLink™ Virus Kit with the iPrep™ Purification Instrument are described below.

## Starting Material

Use this procedure to isolate viral RNA/DNA and bacterial genomic DNA from **200 µL or 400 µL** cell-free samples. See page 6 for sample volume.

## Materials Needed

- Cell-free samples (such as plasma or serum samples, page 6)
- iPrep™ Purification Instrument (page 19)
- iPrep™ Card: Viral DNA/RNA (page 19)
- *Optional:* iPrep™ Small Tips (page 19)
- *Optional:* Carrier RNA (page 9)
- *Optional:* Lysozyme Digestion Buffer (25 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton® X-100) and lysozyme for bacterial gDNA isolation

*Components Supplied with the Kit*

- iPrep™ PureLink™ Virus Cartridge Kit
- iPrep™ Sample and Elution Tubes
- iPrep™ Sample Processing Tubes
- iPrep™ Tips and iPrep™ Tip Holders

## Before Starting

Perform the following before starting:

- Thaw frozen cell-free samples or mix the fresh cell-free samples or lyse bacteria using lysozyme for bacterial gDNA isolation (page 9), and store on ice until use
- Ensure that you have the iPrep™ Card: Viral DNA/RNA (page 19) to run the protocol
- Make sure the iPrep™ Purification Instrument is unpacked and installed

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# Isolating RNA/DNA from Cell-free Samples, Continued

## Elution Volume

The iPrep™ PureLink™ Viral Kit utilizes low, recommended elution volume of 50 µL and 100 µL to elute highly concentrated nucleic acids required for sensitive downstream applications.

You may elute the nucleic acids in an elution volume of 20 µL depending on your downstream application.

**Note:** You need to purchase iPrep™ Small Tips available separately from Invitrogen (page 19) to use 20 µL elution volume.

## Carrier RNA

The iPrep™ PureLink™ Viral Kit protocol does not include carrier RNA during the lysis step as we have not observed any detectable improvement in the performance with carrier RNA.

However, if you wish to include carrier RNA during lysis, use 5.6 µg of carrier RNA (see below for **Sample Lysis**).

## Sample Lysis

The iPrep™ PureLink™ Viral Kit protocol includes an **inline** lysis step, i.e., the sample is placed in the tube on the rack and once the protocol is started, sample lysis occurs in the iPrep™ whereby the Viral Lysis Buffer and Proteinase K are added to the sample and lysis performed at 65ºC for 10 minutes. The inline lysis step reduces sample handling and exposure to any infectious material.

However, if you wish to perform **offline** lysis, i.e., lyse the sample prior to placing samples in the iPrep™, perform lysis using the PureLink™ Viral Lysis Buffer and Proteinase K available separately from Invitrogen (page 19). For best results, use only PureLink™ Viral Lysis Buffer and Proteinase K and not any other lysis buffer for sample lysis.

If you wish to use carrier RNA, use 5.6 µg carrier RNA (Yeast tRNA, available from Invitrogen, page 19) to the samples. Perform offline lysis of samples in the presence of carrier RNA or load the samples with carrier RNA to the iPrep™ Instrument for inline lysis.

**Note:** If you perform offline lysis, the instrument automatically advances to the next step in the protocol and only performs the lysis incubation step.

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# Isolating RNA/DNA from Cell-free Samples, Continued

## Preparing Bacterial Lysates

To isolate bacterial gDNA from cell-free samples, prepare the lysates as follows and load the samples to the iPrep™ Instrument.

**Note:** The need for lysozyme digestion step is dependent on the type of bacteria in use as certain Gram negative bacteria (e.g., *E. coli*) do not need the lysozyme digestion step. If lysozyme digestion step is not performed, load 200 µL or 400 µL bacterial samples to the iPrep™ Instrument.

1. Prepare Lysozyme Digestion Buffer (25 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton® X-100).
2. To 200 µL of Lysozyme Digestion Buffer, add fresh lysozyme to a final concentration of 20 mg/mL.
3. To 200 µL of cell-free sample, add 200 µL 20 mg/mL lysozyme in Lysozyme Digestion Buffer from Step 2. Mix thoroughly.
4. Incubate samples at 37ºC for 30 minutes.
5. Mix briefly again following incubation and add 400 µL sample to the iPrep Sample and Elution Tube (page 11).

## Purification Protocol

Purify viral RNA/DNA and bacterial genomic DNA from cell-free samples using the iPrep™ Purification Instrument as described below.

For details on using the iPrep™ Purification Instrument, refer to the manual supplied with the instrument.

**Insert the iPrep™ Card: Viral DNA/RNA (available separately from Invitrogen, page 19) prior to turning on the instrument.**

1. Ensure the power switch on the iPrep™ Instrument is on the **OFF** position.
2. Open the iPrep™ Card Slot and insert the iPrep™ Card into the slot in the correct orientation (arrow on the card is at the top and card label is facing your left side).
3. Using the power switch located on the left side of the instrument, turn **ON** the instrument.

   If the card is fully inserted in the correct orientation, all axes return to their original positions automatically. The digital display shows the version for the iPrep™ which changes in a few seconds to display the Main menu.

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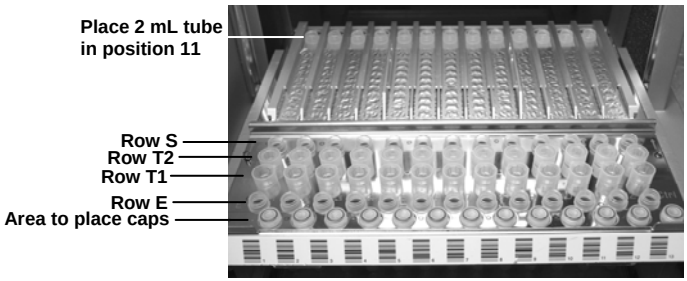
# Isolating RNA/DNA from Cell-free Samples, Continued

**Purification Protocol,** Continued

*Procedure continued from previous page*

4. Press **Start** to run a protocol.
5. Open the iPrep™ instrument door. Remove the iPrep™ Cartridge Rack, and iPrep™ Tip and Tube Rack to set up the platform.
6. Remove the desired number of iPrep™ PureLink™ Virus Cartridges from the box. To collect any solution from the foil, tap the cartridge to deposit the solution at the bottom of the tube.

   **Note:** You can load 1–13 cartridges on the rack depending on the number of samples that you wish to process. If you are loading less than 13 cartridges, ensure that the remaining plastic ware (tips and tubes) are also loaded in the same order as the cartridges.
7. Insert the iPrep™ Sample Processing Tube **in the heated tube position of the cartridge (position 11)** for each of the iPrep™ Virus Cartridge that is used.
8. Load the cartridges on the iPrep™ Cartridge Rack and insert the loaded rack on the iPrep™ platform.
9. Load the iPrep™ Tip and Tube Rack as follows:
   - Load the first row (labeled as **E**) with 1–13 elution tubes **without caps** (you may place the caps on the rack as shown in the figure below)
   - Load the second row (labeled as **T1**) with iPrep™ Small Tips (page 19) in iPrep™ Tip Holders, if you wish to use 20 µL elution volume
   - Load the third row (labeled as **T2**) with iPrep™ Tips in iPrep™ Tip Holders
   - Load the fourth row (labeled as **S**) with iPrep™ Sample and Elution Tubes **containing samples**.

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# Isolating RNA/DNA from Cell-free Samples, Continued

**Purification Protocol,** Continued

*Procedure continued from previous page*

10. Read the sample and elution tube barcode, if needed.
11. Insert the iPrep Tip and Tube rack on the iPrep™ platform as shown on the next page.
12. Close the iPrep™ instrument door.
13. Press **Enter** (↵)to continue.
14. When prompted, select the appropriate lysis mode (inline or offline), sample volume (200 µL or 400 µL), and elution volume (20 µL, 50 µL, or 100 µL).
15. Ensure that you have loaded the cartridges, tubes, and tips in the appropriate positions, and elution tubes **do not have any caps**. **Make sure you have loaded a 2 mL tube in the heated tube position of the cartridge (position 11).**
16. Press **Start**. The automated purification protocol begins and various steps of the protocol including the approximate time remaining are displayed on the digital display.

    **Important: Do not open the door once the protocol has begun.**

    To pause the protocol, press the **Stop** key. To resume the protocol after a pause, press the **Start** key. To cancel/stop the protocol, press the **Stop** key twice. For details, see the iPrep™ Instrument manual.

    The run times are approximate and depend on the parameters chosen such as lysis mode, sample volume, and elution volume as well as the time required to preheat the heat block.
17. At the end of the run, the instrument beeps briefly and digital display shows **Protocol Finished** for 10 seconds. The Main menu appears after 10 seconds.
18. Open the instrument door. Remove and cap the elution tubes containing the purified nucleic acid. Use the RNA/DNA for the desired downstream application or store the purified RNA/DNA at –80°C.
19. Discard the used cartridges, tips, and sample tubes into biohazard waste. **Do not reuse the cartridges.**

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# Isolating RNA/DNA from Cell-free Samples, Continued

**Purification Protocol,** Continued

20. To purify more samples using the same iPrep™ Card, load the racks with new cartridges, tips, and samples, and start the protocol as described above.
21. If you are not using the instrument, close the instrument door and turn the power switch to **OFF**.
22. Remove the iPrep™ Card and store the card in the box, protected from light.

**Analyzing RNA/DNA**

Since the amount of viral RNA/DNA and bacterial gDNA present in cell-free samples 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 and bacterial gDNA using appropriate probes to determine yield or presence of nucleic acids.

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

# Expected Results

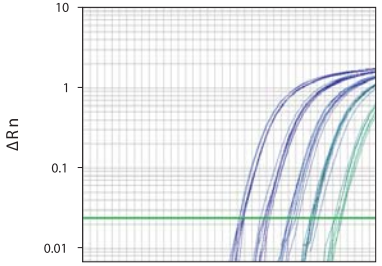
## Adenovirus DNA

Duplicate human plasma samples (400 µL) were spiked with $10^1$, $10^2$, $10^3$, $10^4$, and $10^5$ adenoviral transfection units (TU). Viral DNA was purified using the iPrep™ PureLink™ Viral Kit as described in this manual.

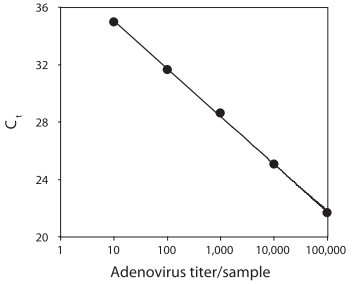
qPCR was performed on the purified DNA (5 µL) using the Platinum® Quantitative PCR SuperMix-UDG w/ROX™ dye (page 19) with TaqMan® primers in an ABI 7900 HT instrument.

**Results:** Detection was linear over a 5-fold range, with sensitivity down to 10 TU per sample.

**Amplification Plot**




**Linearity Plot**

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# Expected Results, Continued

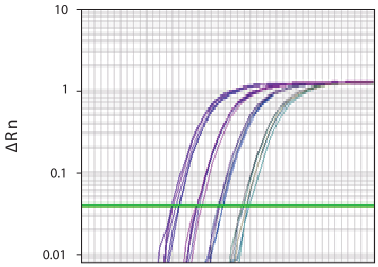
## Lentivirus RNA

Duplicate human serum samples (400 µL) were spiked with $10^1$, $10^2$, $10^3$, $10^4$, and $10^5$ lentivirus transfection units (TU). Viral RNA was purified using the iPrep™ PureLink™ Viral Kit as described in this manual.

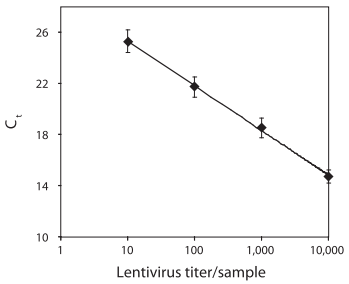
qRT-PCR was performed on the purified RNA(5 µL) using the SuperScript™ III Platinum® One-Step qRT-PCR Kit w/ROX™ dye (page 19) with TaqMan® primers in an ABI 7900 HT instrument.

**Results:** Detection was linear over a 4-fold range, with sensitivity down to 10 TU per sample.

**Amplification Plot**




**Linearity Plot**

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# Expected Results, Continued

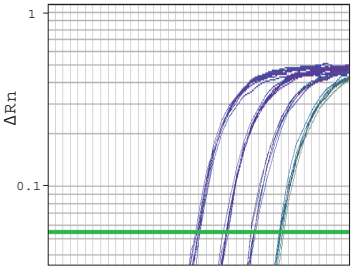
## Bacterial gDNA Results

Duplicate human plasma samples (200 µL) were spiked with $10^2$, $10^3$, $10^4$, and $10^5$ *B. subtilis* colony forming units (CFU). Bacterial gDNA was purified using the iPrep™ PureLink™ Viral Kit as described in this manual using the lysozyme digestion step.

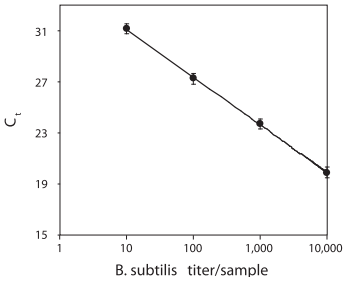
qPCR was performed on the purified DNA (5 µL) using the Platinum® Quantitative PCR SuperMix-UDG w/ROX™ dye (page 19) in an ABI 7900 HT instrument.

**Results:** Detection was linear over a 4-fold range, with sensitivity down to 100 CFU per sample.

**Amplification Plot**




**Linearity Plot**



# Troubleshooting

## Introduction

Refer to the table below to troubleshoot problems with the kit. To troubleshoot problems with the iPrep™ Purification Instrument, refer to the instrument manual.

| Observation | Cause | Solution |
|---|---|---|
| Low nucleic acid yield | Too much starting material used | The purification protocol is designed for use with 200 µL and 400 µL cell-free sample volume. Using greater than the recommended amount of starting material may overload the system and cause clumping which reduces the yield. |
| | Incomplete lysis | Be sure to perform lysis of bacterial samples as described on page 10 prior to loading samples on the iPrep™ Instrument.<br>If you are performing offline lysis, be sure to use PureLink™ Viral Lysis Buffer and Proteinase K (page 19) for best results. |
| | Insufficient amount of Dynabeads® MyOne™ SILANE added | During shipping, some Dynabeads® MyOne™ SILANE solution may adhere to the sealing foil of the cartridge. To collect any bead solution from the foil, tap the cartridge to deposit the bead solution at the bottom of the tube. |
| | 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.<br>Check the quality of the RNA in the original samples using qRT-PCR or RT-PCR. |
| | RNA/DNA 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. |
| No nucleic acid recovered | Magnetic beads stored or handled improperly | • Store cartridge containing the beads at room temperature. Do not freeze the cartridge as the beads may be irreparably damaged.<br>• Make sure that the beads are in solution at all times and do not dry. Dried beads are non-functional. |

# Troubleshooting, Continued

| Observation | Cause | Solution |
|---|---|---|
| Eluate is discolored | Magnetic beads present in the eluate | Remove any magnetic beads using a magnetic separator (MagnaRack™ separator is available from Invitrogen, see page 19) or centrifuge the sample in a microcentrifuge for 1 minute at maximum speed. |
| DNA or RNA is sheared or degraded | Bubbles formed during mixing steps | Make sure that the sample volume is at least 200 µL to prevent excessive bubble formation during mixing. |
| | Purified DNA repeatedly frozen and thawed | Aliquot purified DNA and store at 4°C (short-term) or –20°C (long-term). Avoid repeated freezing and thawing. |
| | DNA contaminated with DNases | Maintain a sterile environment while working (*i.e.* wear gloves and use DNase-free reagents). |
| | RNA contaminated with RNase | Follow the guidelines on page 7 to prevent RNase contamination. |
| Poor performance of nucleic acids in downstream enzymatic reactions | 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 elution volume (20 µL, 50 µL, and 100 µL). |

# Appendix

# Accessory Products

## Additional Products

The table below lists additional products available from Invitrogen for use with the iPrep™ PureLink™ Virus Kit.

For more information, visit www.invitrogen.com or contact Technical Support (page 20).

| Product | Amount | Cat. no. |
|---|---|---|
| iPrep™ Purification Instrument | 1 unit | IS10000 |
| iPrep™ Card: Viral DNA/RNA | 1 card | IS10016 |
| iPrep™ Card: gDNA Blood | 1 card | IS10012 |
| iPrep™ Card: Buffy Coat | 1 card | IS10015 |
| iPrep™ Card: gDNA Tissue | 1 card | IS10013 |
| iPrep™ Card: gDNA Forensic (includes buccal protocol) | 1 card | IS10011 |
| iPrep™ ChargeSwitch® Forensic Kit | 1 kit (52 purifications) | IS10002 |
| iPrep™ ChargeSwitch® Buccal Cell Kit | 1 kit (52 purifications) | IS10003 |
| iPrep™ ChargeSwitch® gDNA Tissue Kit | 1 kit (52 purifications) | IS10004 |
| iPrep™ Small Tips | 1 bag of 52 tips | IS10111 |
| iPrep™ Tip and Tube Rack | 1 rack | IS10101 |
| iPrep™ Cartridge Rack | 1 rack | IS10102 |
| PureLink™ Viral Lysis Buffer | 500 mL | 12282-500 |
| Proteinase K | 5 mL | 25530-049 |
| Yeast tRNA | 25 mg | 15401-011 |
| Quant-iT™ PicoGreen® dsDNA Assay Kit | 1 kit | P7589 |
| Quant-iT™ DNA Assay Kit, High Sensitivity | 1000 assays | Q33120 |
| Quant-iT™ DNA Assay Kit, Broad-Range | 1000 assays | Q33130 |
| Qubit® Fluorometer | 1 each | Q32857 |
| MagnaRack™ Magnetic Separator | 1 rack | CS15000 |
| Platinum® Quantitative PCR SuperMix-UDG w/ROX | 100 reactions | 11743-100 |
| SuperScript® III One-Step RT-PCR System with Platinum® *Taq* DNA Polymerase | 100 reactions | 12574-026 |
| RNase *AWAY*™ Reagent | 250 ml | 10328-011 |

# Technical Support

## World Wide Web

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

## Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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| Carlsbad, CA 92008 USA | 3 Fountain Drive |
| Tel: 1 760 603 7200 | Paisley PA4 9RF, UK |
| Tel (Toll Free): 1 800 955 6288 | Tel: +44 (0) 141 814 6100 |
| Fax: 1 760 602 6500 | Tech Fax: +44 (0) 141 814 6117 |
| E-mail: tech_support@invitrogen.com | E-mail: eurotech@invitrogen.com |

*Continued on next page*

# Technical Support, Continued

## SDS Requests

SDSs (Safety Data Sheets) are available on our website at www.invitrogen.com/sds.

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# Notes

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