USER GUIDE

invitrogen
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PureLink™ Viral RNA/DNA Kit

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

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Table of Contents

Experienced Users' Viral Kit Procedure

Introduction

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

Step	Action
Preparing Lysates	This lysate preparation protocol is suitable for **200 µL** starting material. To process >200 µL (up to 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:** For <200 µL of 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** the lysate to the Viral Spin Column (in a collection tube). 2. Centrifuge the column at 6,800 × 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 6,800 × 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.

Kit Contents and Storage

Shipping and Storage

All components of the PureLink™ Viral RNA/DNA Kit 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.

Contents

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

Note: Since the Viral Kit is designed for purifying samples using a starting volume of up to 500 µL, some reagents in the kit maybe provided in excess in the amount needed.

Component	12280-101
Viral Lysis Buffer (L22)	160 mL
Wash Buffer II (5X)	75 mL
Proteinase K (20 mg/mL) in storage buffer (proprietary)	16 mL
Carrier RNA (lyophilized)	5 × 310 µg
Sterile, RNase-free Water (E3)	75 mL
Viral Spin Columns with Collection Tubes	5 × 50
Wash Tubes (2.0 mL)	5 × 50
Recovery Tubes (1.5 mL)	5 × 50
Adenovirus	60 µL

Introduction

About the Kit

Kit Usage

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

The kit provides a method to purify viral RNA/DNA from fresh or frozen cell-free biological fluids (plasma, serum, cerebrospinal fluid) and cell culture supernatants. The purified viral RNA/DNA is suitable for use in downstream applications for viral detection and genotyping.

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 up to 500 µL cell-free samples within 45 minutes
- Ability to elute viral nucleic acids in low volumes (10–50 µL) to allow sensitive downstream analysis
- Purified nucleic acid is free of contaminants such as proteins and nucleases
- Reliable performance of the purified viral nucleic acids in downstream applications

System Overview

Viral particles in cell-free samples are lysed with Proteinase K and Lysis Buffer (L22) containing Carrier RNA at 56ºC. The Lysis Buffer (L22) is specifically formulated to allow efficient 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. The viral RNA/DNA molecules bind to the silica-based media under highly denaturing conditions 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

Carrier RNA

The Carrier RNA included with the PureLink™ Viral RNA/DNA Kit is yeast tRNA (page 14). The presence of an excess amount of Carrier RNA in relation 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 of Carrier RNA for 200–500 µL of sample. Most of the Carrier RNA is removed during the purification process because it is <200 bp. Any remaining Carrier RNA does not interfere with downstream applications, however, depending on your application, you may validate the assay using less Carrier RNA.

Proteinase K

Proteinase K is used for efficient lysis of viral particles. It is active under the 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

Viral Kit Specifications

Starting Material:	Up to 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

Methods

Before Starting

Introduction

Review the information in this section before starting. Guidelines are included for the recommended amount of starting material for use 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 the 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 plasticware
- 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 14) to remove RNase contamination from surfaces

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Before Starting, Continued

Starting Material

The PureLink™ 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 (such as plasma or serum) and proceed immediately to the purification protocol (page 6). 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 PureLink™ Viral RNA/DNA Kit can process sample volumes of up to 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.

Preparing Wash Buffer

Add 300 mL 96–100% ethanol to 75 mL Wash Buffer (W5) included with the kit.

Continued on next page

Preparing Carrier RNA

The recommended purification protocol uses 5.6 µg Carrier RNA per sample (for up to 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 with the kit) to obtain a 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 formulas below:

   N × 0.21 mL = 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 solution
4. Thaw the calculated amount of 1 µg/µL Carrier RNA stock solution.
5. Add the calculated volume of Carrier RNA stock solution (B, above) to the calculated volume of Lysis Buffer (A, above) in a sterile tube. Mix gently by pipetting. 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:

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

2.1 mL × 28 µL/mL = 58.8 µL (volume of Carrier RNA)

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).

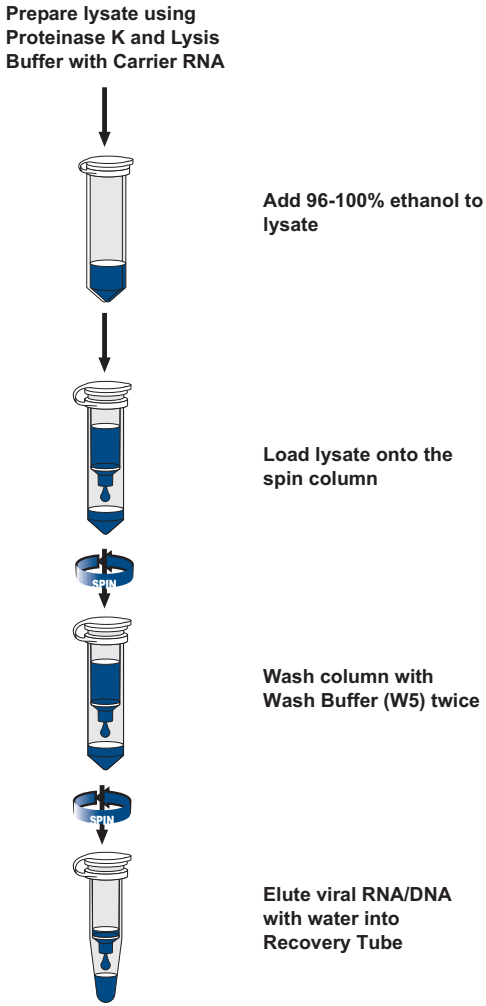
Purification Procedure for Viral Kit

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 Kit is shown below.



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Purification Procedure for Viral Kit,
Continued

Follow the recommendations below to obtain the best results:

- Perform all centrifugation steps at room temperature
- Be sure Wash Buffer (W5) contains ethanol (page 4)
- Perform the recommended wash steps to obtain high-quality RNA
- Always pipet water to the center of the Viral Spin Column and incubate for 1 minute before elution

- 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 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.

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Purification Procedure for Viral Kit,
Continued

Materials Needed

- Cell-free samples (such as plasma or serum samples)
- Appropriate amount of Lysis Buffer (L22) containing Carrier RNA, page 5
- 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 described below is scaled for **200 µL of** starting material. To process >200 µL (up to 500 µL) sample volume, scale the reagent volumes accordingly.

Note: The amount of Carrier RNA does not have to be scaled-up. Use up to 5.6 µg Carrier RNA per sample for volumes up to 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 the 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). Cap the tube and vortex 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**, next page.

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Purification Procedure for Viral Kit,
Continued

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 lysate with ethanol (~675 µL) onto the Viral Spin Column.
5. Close the lid and centrifuge the column at ~6,800 × g for 1 minute. Discard the collection tube and flow-through.

   Note: If you are processing >200 µL starting material, you need to perform multiple loading of the lysate by transferring any remaining lysate to the same Viral Spin Column (above) and centrifuge at 6,800 × g for 1 minute.
6. Place the spin column in a clean 2 mL Wash Tube (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 ~6,800 × 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 14), 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 ~6,800 × 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**, next page.

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Purification Procedure for Viral 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 up to 150 µL for elution (page 7).
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 Viral Kit

Results

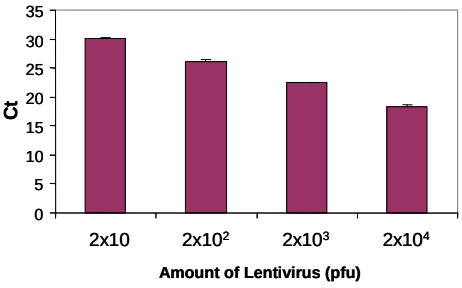
Examples of results obtained after purification of viral RNA/DNA using the PureLink™ Viral RNA/DNA Kit are shown below.

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 Kit as described in this manual. Elution was performed with 50 µL RNase-free water.

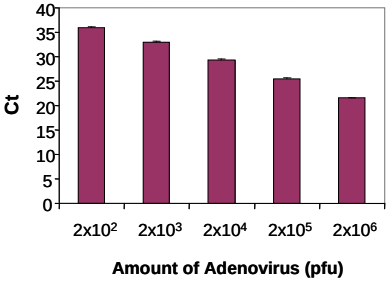
For lentivirus RNA sample, 5 µL of the purified RNA was used to perform qRT-PCR using the SuperScript™ III Platinum® One-Step qRT-PCR Kit w/ROX with TaqMan® primers in an ABI 7700 instrument. For adenovirus DNA sample, 5 µL of purified DNA was used to perform qPCR using the Platinum® Quantitative PCR SuperMix-UDG w/ROX kit (page 14) 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 Kit.

Lentivirus RNA



Adenovirus DNA



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 Viral Kit, adjust the reagent volumes accordingly to obtain complete lysis. If cryoprecipitates are visible in frozen viral samples, remove the cryoprecipitates by centrifugation (page 4) 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 5). Once the Carrier RNA is reconstituted in water, aliquot the Carrier RNA and store at –20ºC. 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 4. 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.

Continued on next page

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 3 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 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 up to 500 µL sample volume. **Do not** increase the Carrier RNA amount when processing 500 µL sample volume using the Viral Kit.

Appendix

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 14).

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

World Wide Web

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

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

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