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PureLink™ Viral RNA/DNA Mini Kit

USER GUIDE

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

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A.0	16 June 2016	Updated Wash Buffer name from W5 to WII; Removed Cat. No. 12280096
2.0	27 September 2012	Baseline for this revision history

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Experienced users' 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
Prepare 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.
Purify RNA/DNA	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 (WII) with ethanol. Centrifuge at 6800 × *g* for 1 minute. Discard the flow through. 4. **Repeat** wash Step 3 with 500 µL Wash Buffer (WII) 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 (WII). 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.

Contents and storage

Shipping and storage

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

Types of kits

This manual supports the following kit.

Kit	Reactions	Cat. No.
PureLink™ Viral RNA/DNA Mini Kit	50	12280050

Contents

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

Note: Since the 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.

Component	12280050
Viral Lysis Buffer (L22)	32 mL
Wash Buffer (WII) (5X)	15 mL
Proteinase K (20 mg/mL) in storage buffer (proprietary)	2 × 1.6 mL
Carrier RNA (lyophilized)	310 µg
Sterile, RNase-free Water (E3)	15.5 mL
Viral Spin Columns with Collection Tubes	50 each
Wash Tubes (2.0-mL)	2 × 50
Recovery Tubes (1.5-mL)	50 each

Product information

Overview

Introduction

The PureLink™ Viral RNA/DNA Mini 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™ 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 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 PureLink™ Viral RNA/DNA Mini 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 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. 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.

Overview, continued

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
- 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 Mini Kit is yeast tRNA (page 20). 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

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

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! 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 **thermofisher.com/support**.

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 20) to remove RNase contamination from surfaces

Before starting, continued

Starting material

The PureLink™ Viral RNA/DNA Mini 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 12). 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.

Prepare Wash Buffer

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

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

Prepare 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).

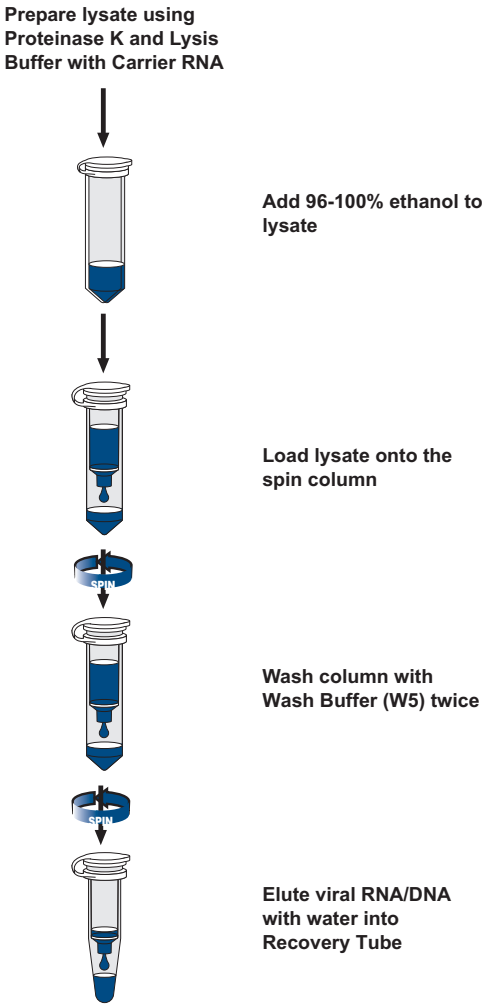
Purification procedure

Introduction

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

Workflow

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



Purification procedure, continued

Procedural guidelines

Follow the recommendations below to obtain the best results:

- Perform all centrifugation steps at room temperature
- Be sure to add ethanol to Wash Buffer (WII) before use (page 10)
- 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 precautions

- 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, continued

Required materials not supplied

Components supplied by the user

- Cell-free samples (such as plasma or serum samples)
- Appropriate amount of Lysis Buffer (L22) containing Carrier RNA, page 11
- Heat block set to 56ºC
- 96–100% ethanol (BP8202 or BP2818, available from **fishersci.com**, or equivalent)
- 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 (WII)
- Sterile, RNase-free Water (E3)
- Viral Spin Column in Collection Tubes
- Wash Tubes and Recovery Tubes

Prepare 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 15.

Bind and wash RNA/DNA

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 (WII) 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 20), if you do not wish to reuse the Wash Tube.
8. Add 500 µL Wash Buffer (WII) 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 16.

Purification procedure, continued

Elute RNA/DNA

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 13).
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.

Analyze 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

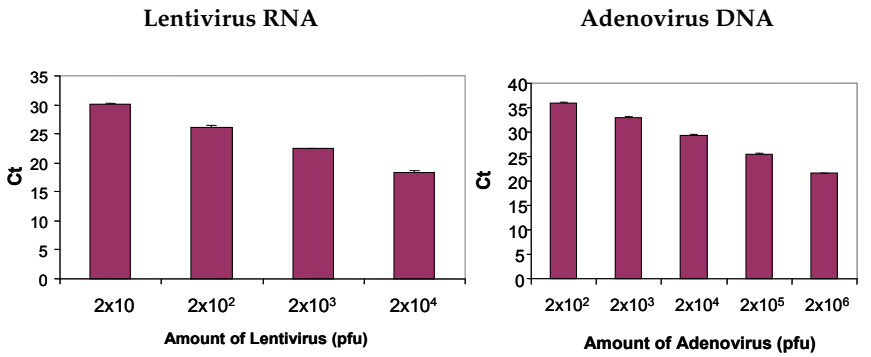
Results

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 20) 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.



Troubleshooting

Observation	Possible cause	Recommended action
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. If cryoprecipitates are visible in frozen viral samples, remove the cryoprecipitates by centrifugation (page 10) 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 11). 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 (WII)	Be sure to add 96–100% ethanol to Wash Buffer (WII) as described on page 10. 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.

Observation	Possible cause	Recommended action
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 9 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 (WII) can inhibit downstream enzymatic reactions. To remove Wash Buffer (WII), discard Wash Buffer (WII) 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

All materials are available through **thermofisher.com**.

Item	Quantity	Cat. No.
PureLink™ Viral Collection Tubes	100	12282100
RNase *AWAY*™ Decontamination Reagent	250 mL	10328011
PureLink™ Foil Tape	50 pieces	12261012
PureLink™ Viral Lysis Buffer (L22)	500 mL	12282500
UltraPure™ DEPC-treated Water	1 L	750023
UltraPure™ DNase/RNase-Free Distilled Water	500 mL	10977015
Yeast tRNA	25 mg	15401011
Phosphate Buffered Saline (PBS), 1X	500 mL	10010023
SuperScript™ III One-Step RT-PCR System with Platinum™ *Taq* DNA Polymerase	100 reactions	12574026
RNA UltraSense™ One-Step Quantitative RT-PCR System	100 reactions	11732927
Platinum™ Quantitative PCR SuperMix-UDG w/ROX	100 reactions	11743100

Technical support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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