USER GUIDE

applied biosystems
by life technologies™

MagMAX™ Viral RNA Isolation Kit

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life technologies™

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Part Number 1939M Rev. E
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About This Guide

WARNING! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information the "Safety" appendix in this document.

Purpose

The *MagMAX™ Viral RNA Isolation Kit User Guide* provides detailed procedures, reference information and troubleshooting for the kit.

User attention words

Five user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation or accurate chemistry kit use.

CAUTION! Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Except for IMPORTANTs, the safety alert words in user documentation appear with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instrument. See the "Safety" appendix for descriptions of the symbols.

MagMAX™ Viral RNA Isolation Kit

Introduction

Product Description

The MagMAX™ Viral RNA Isolation Kit is designed for rapid purification of viral RNA and DNA from biofluid samples such as serum, plasma, nasal fluid, milk, and swab samples. For viral nucleic acid isolation from whole blood or cell culture and tissue samples we recommend the MagMAX-96 Blood Kit and MagMAX-96 Total RNA Isolation Kit.

The microspherical paramagnetic beads used in the kit have a large available binding surface and can be fully dispersed in solution, allowing thorough nucleic acid binding, washing, and elution. The procedure, therefore, delivers very consistent yields of high quality RNA and DNA with little sample-to-sample variation.

Overview of the Procedure

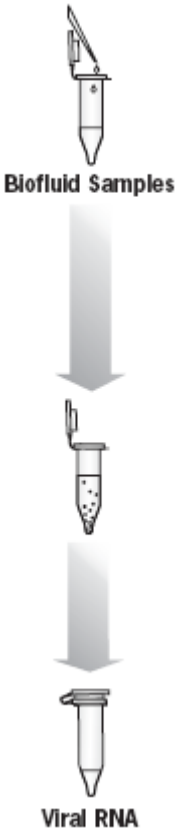
The MagMAX Viral RNA Isolation Kit employs a classic method for disrupting samples in a guanidinium thiocyanate-based solution that rapidly releases viral RNA and DNA while simultaneously inactivating nucleases in the sample matrix (Chirgwin, et al., 1979; Chomczynski and Sacchi, 1987). Paramagnetic beads with a nucleic acid binding surface are then added to the sample to bind nucleic acids. The beads/nucleic acids are captured on magnets, and proteins and other contaminants are washed away. The beads are then washed again to remove residual binding solution. Nucleic acids are eluted in a small volume of elution buffer. Note that this procedure recovers total nucleic acids, so if cells are present in the sample, cellular DNA/RNA will be recovered along with the viral RNA.

Note: For high throughput viral RNA and DNA isolation, we recommend using the MagMAX-96 Viral RNA Isolation Kit (Cat #AM1836).

The MagMAX Viral RNA Isolation Kit can efficiently isolate viral RNA and DNA from samples as large as 400 µL. RNA recovery is dependant upon sample type and is typically greater than 75%. The RNA recovered with the kit is of high quality and purity, and is suitable for real-time RT-PCR†. Figure 2 shows an example of qRT-PCR data that illustrates linear RNA recovery using several different sample types with a broad range of RNA inputs.

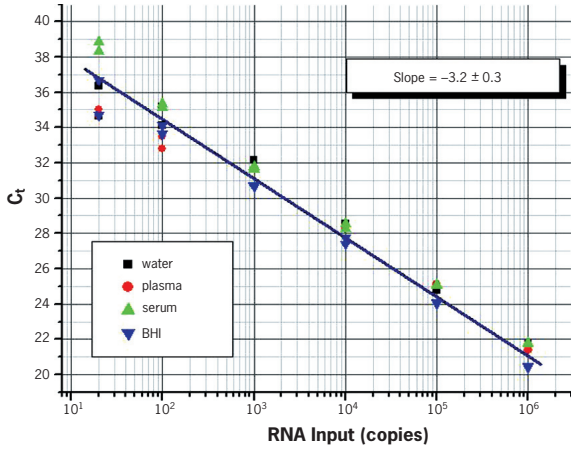
† This product is compatible with the 5' nuclease detection and dsDNA-binding dye processes covered by patents owned or licensable by Life Technologies. No license under these patents is conveyed expressly, by implication, or by estoppel to the purchaser by the purchase of this product. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Life Technologies, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Figure 1 MagMAX™ Viral RNA Isolation Kit Procedure Overview



1. "Add 400 µL sample to 802 µL Lysis/Binding Solution" on page 11
2. "Add 20 µL Bead Mix; mix for 4 min" on page 12
3. "Capture the RNA Binding Beads and carefully discard the supernatant" on page 12
4. "Wash twice with 300 µL Wash Solution 1" on page 12
5. "Wash twice with 450 µL Wash Solution 2" on page 12
6. "Dry the beads by leaving the tube open on the bench for 2 min" on page 13
7. "Elute the RNA/DNA in 50 µL Elution Buffer" on page 13

Figure 2 Linear Recovery of Viral RNA Using MagMAX™ Viral RNA Isolation Kit. Serial dilutions of HIV Armored RNA® transcripts were spiked into water, plasma, serum, and BHI (brain heart infusion broth), and viral RNA was isolated using the MagMAX Viral RNA Isolation Kit according to the protocol. Equivalent volumes of recovered viral RNA (1/4 of eluted volume) were used in a 25 µL qRT-PCR to detect the HIV transcript.



Kit Components and Storage Conditions

The MagMAX Viral RNA Isolation Kit contains reagents to isolate RNA from 50 samples.

Amount		Component	Storage
50		Processing Tubes	room temp
44	mL	Lysis/Binding Soln Concentrate See step 2. on page 10 before use	room temp†
36	mL	Wash Solution 1 Concentrate (Add 12 mL 100% isopropanol before use)	room temp
55	mL	Wash Solution 2 Concentrate (Add 44 mL 100% ethanol before use)	4°C or room temp
5	mL	Elution Buffer	4°C or room temp
550	µL	RNA Binding Beads	4°C†
110	µL	Carrier RNA	–20°C
550	µL	Lysis/Binding Enhancer	–20°C

† Do not freeze these kit components.

MagMAX Viral RNA Isolation Kit Protocol

Equipment and Reagent Preparation

1. RNase precautions

Lab bench and pipettors

Before working with RNA, it is always a good idea to clean the lab bench and pipettors with an RNase decontamination solution (e.g., Ambion RNaseZap® Solution).

Gloves and RNase-free technique

Wear laboratory gloves for this procedure; they protect you from the reagents, and they protect the RNA from nucleases that are present on skin.

Use RNase-free pipette tips to handle the kit reagents, and avoid putting used tips into the reagent containers.

2. Add Carrier RNA to the Lysis/Binding Solution Concentrate, mix, then add isopropanol

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IMPORTANT! Shipment on dry ice may cause the Carrier RNA to become gelatinous and difficult to pipet. If you experience problems when attempting to pipet the Carrier RNA, we recommend heating it in a hybridization oven at 37°C for 10–15 min. A heat block may be used for this incubation, as long as the block accommodates the tube to uniformly heat the solution to 37°C. After heating, vortex vigorously, then spin briefly; you should now be able to easily pipette the solution accurately.
Prepared Lysis/Binding Solution is stable at room temperature for one month. Prepare only the amount of Lysis/Binding Solution needed for one month by scaling the reagent volumes down proportionally if necessary.
We do not recommend storing the prepared Lysis/Binding Solution at 4°C or below as this may cause the Carrier RNA to precipitate; if the solution is inadvertently stored at 4°C, warm it at 37°C and shake to dissolve any precipitates before use.

Add Carrier RNA to Lysis/Binding Solution Concentrate according to the table below, and mix briefly. Then add 100% isopropanol and mix well. This mixture is called Lysis/Binding Solution in these instructions.

Note: If you have an internal positive control RNA to monitor viral RNA purification and detection, we recommend adding it to the Lysis/Binding Concentrate before adding the isopropanol.

We recommend including ~10% overage to cover pipetting error when preparing the Lysis/Binding Solution. Store at room temperature.

a. Combine the following:	**Per Rxn**		**10 Rxn**		**Entire Bottle**	
Lysis/Binding Soln. Concentrate	400	µL	4.4	mL	22	mL
Carrier RNA	2	µL	22	µL	110	µL
b. Mix briefly, then add:						
100% Isopropanol	400	µL	4.4	mL	22	mL
c. Mix well by vortexing.						

3. Add 12 mL 100% isopropanol to Wash Solution 1 Concentrate

   Add 12 mL 100% isopropanol to the bottle labeled Wash Solution 1 Concentrate and mix well. Mark the label to indicate that the isopropanol was added. Store at room temperature.

   The resulting mixture is called Wash Solution 1 in these instructions.

4. Add 44 mL 100% ethanol to Wash Solution 2 Concentrate

   Add 44 mL 100% ethanol to the bottle labeled as Wash Solution 2 Concentrate and mix well. Mark the label to indicate that the ethanol was added.

   Store at room temperature.

   The resulting mixture is called Wash Solution 2 in these instructions.

5. Prepare Bead Mix

   Each isolation reaction requires 20 µL of Bead Mix. Although the mixture is stable at 4°C for up to 2 weeks, we recommend preparing Bead Mix on the day it will be used.

   a. Vortex the RNA Binding Beads at moderate speed to form a uniform suspension before pipetting.

   b. Prepare Bead Mix by combining the volumes of RNA Binding Beads and Lysis/Binding Enhancer shown in the table below appropriate for the number of isolation reactions to be performed that day. Mix thoroughly by gently vortexing.

      We recommend including ~10% overage to cover pipetting error when preparing the Bead Mix.

Component	Per Reaction		10 Rxns (+10%)		50 Rxns (+10%)	
RNA Binding Beads	10	µL	110	µL	550	µL
Lysis/Binding Enhancer	10	µL	110	µL	550	µL

   c. Place the prepared Bead Mix on ice until it is needed.

RNA Isolation Protocol

Sample type

This kit is designed for isolation of viral RNA and DNA from cell-free, or nearly cell-free samples. For example, biological fluids such as serum, plasma, milk, urine, meconium, and nasal fluids can be used with the kit. Other common sample types such as spent culture medium and swab samples are also compatible.

Sample volume

With up to 400 µL sample input, the MagMAX Viral RNA Isolation procedure can be completed in the 1.5 mL tubes provided in the kit. It is possible to process >400 µL samples in a larger tube by proportionally scaling up the reaction; however, additional reagents, larger tubes, and an appropriate magnetic stand are required.

1. Add 400 µL sample to 802 µL Lysis/Binding Solution

   a. Pipet 802 µL prepared Lysis/Binding Solution (Carrier RNA and isopropanol added) into each Processing Tube.

b. Transfer up to 400 µL of sample to the Lysis/Binding Solution in the Processing Tube.

   When adding sample, immerse pipette tips slightly in the Lysis/Binding Solution to prevent creating aerosols that can lead to cross-contamination.

c. Mix by gently vortexing for 30 sec or by flicking the tube several times. Then centrifuge briefly (~2 sec) to collect the contents at the bottom of the tube.

2. Add 20 µL Bead Mix; mix for 4 min

   a. Vortex the Bead Mix at moderate speed to create a uniform suspension before pipetting (prepared as described in step 5. on page 11).

   b. Add 20 µL Bead Mix to each sample.

   c. Gently shake the sample for 4 min on a vortex mixer to fully lyse viruses and bind RNA to the RNA Binding Beads.

   d. Centrifuge briefly (~2 sec) to collect tube contents.

3. Capture the RNA Binding Beads and carefully discard the supernatant

   a. Move the Processing Tube to a magnetic stand to capture the RNA Binding Beads. Leave the tube on the magnetic stand for at least 3 min. When capture is complete, the RNA Binding Beads will form a pellet against the magnet in the magnetic stand. The capture time depends on the magnetic stand used.

   b. Carefully aspirate and discard the supernatant without disturbing the beads, and remove the Processing Tube from the magnetic stand.

   IMPORTANT! To obtain pure RNA, it is important to completely remove the supernatant at this step.

4. Wash twice with 300 µL Wash Solution 1

   a. Add 300 µL Wash Solution 1 (isopropanol added) to each sample and vortex at moderate speed for 30 sec.

   b. Centrifuge briefly (~2 sec) to collect tube contents.

   c. Capture the RNA Binding Beads on a magnetic stand for 3–5 min, or until the mixture becomes clear, indicating that capture is complete.

   d. Carefully aspirate and discard the supernatant without disturbing the beads and remove the Processing Tube from the magnetic stand.

      It is critical to move the Processing Tube off the magnetic stand before the subsequent wash.

   e. Repeat above steps to wash a second time with 300 µL Wash Solution 1.

5. Wash twice with 450 µL Wash Solution 2

   a. Add 450 µL Wash Solution 2 (ethanol added) to each sample and vortex at moderate speed for 30 sec.

   b. Centrifuge briefly (~2 sec) to collect tube contents.

   c. Capture the RNA Binding Beads as in the previous wash.

   d. Carefully aspirate and discard the supernatant without disturbing the beads and remove the Processing Tube from the magnetic stand. As in the previous wash, it is critical to move the Processing Tube off the magnetic stand before the subsequent wash.

e. Repeat above steps to wash with a second 450 µL of Wash Solution 2. It is important to remove as much Wash Solution 2 from the samples as possible, because otherwise it could inhibit downstream applications such as RT-PCR.

IMPORTANT! To obtain pure RNA, it is important to completely remove the supernatant at this step.

6. Dry the beads by leaving the tube open on the bench for 2 min
   a. Leave the tube open at room temp for 2 min to allow any remaining alcohol from the Wash Solution 2 to evaporate.
   b. Inspect the tubes and if there is residual solution, remove as much as possible with a very fine-tipped pipettor. Then leave the tube open on the bench for another minute.
7. Elute the RNA/DNA in 50 µL Elution Buffer
   a. Add 50 µL Elution Buffer (room temp or warmed to 37–65°C) to each sample, and shake or vortex vigorously for 4 min.

      Note: The elution volume is somewhat flexible; RNA/DNA can be eluted as little as 20 µL, or in >50 µL to achieve the desired final nucleic acid concentration. The volume of Elution Buffer supplied with the kit is enough for 50 samples at 100 µL each.

   b. Capture the RNA Binding Beads as in the previous steps. ***The purified RNA will be in the supernatant.***
   c. Transfer the supernatant, which contains the RNA, to a nuclease-free container appropriate for your application, and store the purified RNA at –20°C.

Analyzing Viral RNA and DNA

Detect viral RNA by real-time RT-PCR and detect DNA by real-time qPCR

This kit is designed for purification of RNA for RT-PCR amplification, and purification of DNA for qPCR. Quantitative real-time RT-PCR/ PCR is a powerful method for viral RNA/DNA detection and is the recommended analysis tool.

Quantitate Carrier RNA recovered

The viral RNA recovered from most samples will be present in very limited amounts; the majority of RNA in the purified sample will be the Carrier RNA that was added to the Lysis/Binding Solution. RNA recovery is heavily dependent upon sample type (e.g., plasma vs. swab samples). With most sample types, up to 75% of the carrier RNA should be recovered. Using the recommended volume (802 µL) of prepared Lysis/ Binding Solution, each sample will contain approximately 2 µg of Carrier RNA; therefore >10 ng/µL RNA should be recovered.

Quantitate the amount of Carrier RNA by UV absorbance at 260 nm (A_{260}). If you are using a NanoDrop® 1000 Spectrophotometer, 1.5 µL of nucleic acid solution can be measured without dilution.

Alternatively, the RNA concentration can be determined by diluting an aliquot of the preparation in TE (10 mM Tris-HCl, pH 8; 1 mM EDTA) and reading the absorbance in a traditional spectrophotometer at 260 nm. Find the concentration in µg/mL by multiplying the A_{260} by the dilution factor and the extinction coefficient. (1 = 40 µg RNA/mL).

$$A_{260} \text{ X dilution factor X } 40 = \mu g \text{ RNA/mL}$$

Troubleshooting

Poor Viral Nucleic Acid Detection

If poor or no viral RNA or DNA signal is observed by RT-PCR (i.e., the C_t is higher than expected), it could be due to inhibitors in the nucleic acid recovered or poor nucleic acid recovery.

Inhibitors of RT-PCR

With most samples, the MagMAX Viral RNA Isolation protocol yields very pure nucleic acid; however, with samples that contain excessively high amounts of reaction inhibitors, enough may be carried over to affect RT-PCR.

- Minimize the effect of inhibitors by using less RNA or DNA in the reaction

  The effect of inhibitors can be minimized or eliminated by reducing the amount of RNA used in qRT-PCR or the amount of DNA used in qPCR. Try diluting the eluted nucleic acid 10-fold and repeating the RT-PCR or PCR; if a signal is observed using the diluted sample, this would indicate the presence of inhibitors in the eluted nucleic acid.
- Detecting protein contamination

  The UV absorbance at 260 and 280 nm can be used to determine if there is protein contamination in the sample. Proteins have an absorbance peak at ~280 nm, whereas nucleic acids have an absorbance peak at ~260 nm. The ratio of A_{260}/A_{280} should be ~2.0 for pure nucleic acid isolated from cell-free samples with the MagMAX Viral RNA Isolation Kit. An A_{260}/A_{280} ratio below this is indicative of protein carryover.

Poor RNA or DNA recovery: assess recovery of the Carrier RNA

- Expected Carrier RNA recovery

  Using the MagMAX Viral RNA Isolation Kit protocol, 35–75% of the input RNA should be recovered (recovery is heavily dependent upon sample type). Using the recommended volume (802 µL) of prepared Lysis/Binding Solution, each sample will contain approximately 2 µg of Carrier RNA; therefore >10 ng/µL RNA should be recovered. Instructions for quantitating Carrier RNA are found in section "Quantitate Carrier RNA recovered" on page 13. Be aware that any cellular DNA or RNA in the prep will lead to an overestimation of yield, since all nucleic acids absorb at 260 nm.

  Since the Carrier RNA is in great excess relative to viral RNA and DNA obtained from the sample, the contribution of viral nucleic acid to the absorbance is negligible. However, sample types containing small numbers of cells, such as urine and milk, will contain fairly significant amounts of cellular RNA and DNA.
- Good recovery of Carrier RNA, but viral RNA or DNA cannot be detected

If the Carrier RNA was recovered at expected levels (>10 ng/µL RNA), but viral nucleic acid cannot be detected using a proven qRT-PCR or qPCR assay system, this would suggest the absence of virus in the original sample, poor lysis of viral particles, or problems with the RT-PCR or PCR. Consider the recommendation in the previous section for diluting your sample to minimize the effects of inhibitors.

Troubleshoot viral particle lysis: If diluting the viral nucleic acid does not help, the problem is most likely due to incomplete lysis of viral particles. Consider increasing the incubation time of the lysis/binding in step 1.c. on page 12 to 10–15 min.

- Lower-than-expected Carrier RNA recovery

  Poor recovery of the Carrier RNA (≤10 ng/µL) could indicate a problem with the nucleic acid isolation process. See section "Sample-to-Sample Variation in RNA/DNA Yield " on page 15 below for suggestions that may help with nucleic acid recovery. If these suggestions do not improve Carrier RNA recovery, the procedure may require further optimization for use with different sample types; contact Life Technologies's Technical Services Department for more information on how to optimize the kit for use with various sample types.

Sample-to-Sample Variation in RNA/DNA Yield

The Carrier RNA yield should be fairly uniform among samples of the same type. However, the efficiency of RNA/DNA recovery may differ between different matrices. If large variations in nucleic acid yield are observed, consider the following suggestions:

RNA Binding Beads were not fully resuspended/dispersed

In general, the RNA Binding Beads will disperse more easily when the temperature of the mixture is warmer than 20°C.

a. Make sure the Bead Mix is fully resuspended before adding it to the Processing Tube at the start of the procedure.

b. Make sure that the RNA Binding Beads are fully resuspended in Elution Buffer to efficiently elute nucleic acids from the beads. Fully resuspended beads will produce a homogenous brown solution. If the solution is clear, with brown clumps, it means that the beads are not fully resuspended. Preheating the Elution Buffer to 60–65°C just before use will facilitate resuspension of the beads.

c. Avoid overdrying the RNA Binding Beads before eluting the RNA/DNA because this may make the beads more difficult to resuspend. If the beads are inadvertently overdried, increase the mixing time (to 10 min) during the elution step to allow the beads to rehydrate.

RNA Binding Beads were unintentionally lost

Since the principle of this procedure is to immobilize nucleic acids on the RNA Binding Beads, any loss of beads during the procedure will result in loss of RNA/DNA. Avoid aspirating the RNA Binding Beads when removing supernatant from the captured beads. To determine whether RNA Binding Beads have been inadvertently aspirated with supernatant, it may be helpful to collect all supernatants (except the final RNA-containing supernatant) in a single container. Observe the color of the collected supernatant. If RNA Binding Beads are in the supernatant, they will color it light brown.

To prevent aspiration of RNA Binding Beads in subsequent experiments, observe the following precautions:

- Use sufficient magnetic capture time.
- Aspirate supernatant slowly.
- Keep pipette tip openings away from the captured RNA Binding Beads when aspirating supernatant.

RNA Binding Bead Carryover

If RNA Binding Beads are carried over into the eluate, they will cause the solution to be light brown in color. A small quantity of beads in the sample does not inhibit RT reactions or RT-PCR.

- See section "RNA Binding Beads were unintentionally lost" on page 15 for suggestions for avoiding bead carryover.
- To remove RNA Binding Beads from RNA samples, place the Processing Tube on a magnetic stand to capture the beads for ~1 min. Then transfer the nucleic acid solution(s) to a fresh nuclease-free plate or tubes.

A Materials Not Included with the Kit

Required Materials Not Provided With the Kit

Reagents

- 100% ethanol, ACS grade or better
- 100% isopropanol, ACS grade or better

Equipment

- A vortex mixer (optional: a vortex adapter for hands-free mixing of several samples simultaneously, e.g., Cat #AM10024).
- Magnetic stand for 1.5 mL tubes, e.g., the Ambion 6 Tube Magnetic Stand (Cat #AM10055)

Related Products Available from Life Technologies

RNaseZap® Solution Part nos. AM9780, AM9782, AM9784	RNaseZap RNase Decontamination Solution is simply sprayed, poured, or wiped onto surfaces to instantly inactivate RNases. Rinsing twice with distilled water will eliminate all traces of RNase and RNaseZap Solution.
6 Tube Magnetic Stand Part no. AM10055	Designed to hold up to six standard microcentrifuge tubes, the 6 Tube Magnetic Stand houses a strong magnet for fast, efficient separation of magnetic beads from solutions.
MagMAX™-96 Viral RNA Isolation Kit Part no. AM1836	The MagMAX™-96 Viral RNA Isolation Kit is designed for rapid high throughput purification of viral RNA in 96-well plates from biological fluids and cell-free samples such as serum, plasma, swabs, and cell culture media. As few as 20 copies of viral RNA can be detected from 50 µL of input sample.
MagMAX™-96 Blood RNA Isolation Kit Part no. AM1837	The MagMAX™-96 Blood RNA Isolation Kit is a magnetic bead-based system designed for rapid high throughput isolation of total and viral RNA in 96-well plates from mammalian whole blood and milk. The recovered viral RNA or total RNA can be used directly for quantitative reverse transcriptase PCR (qRT-PCR) for viral veterinary molecular diagnostics or gene expression profiling, respectively.
MagMAX™-96 Total RNA Isolation Kit Part no. AM1830	The MagMAX™-96 Total RNA Isolation Kit is a magnetic bead based total RNA purification system designed for rapid high throughput processing of cells in 96-well plates. High yield and high quality total RNA can be obtained from 100 to 500,000 cultured eukaryotic cells. The kit can also be used for total RNA isolation from small tissue samples.
MessageSensor™ RT Kit Part no. AM1745	The MessageSensor RT Kit for one-step qRT-PCR includes an optimized set of reagents for exceptionally sensitive reverse transcription. The kit is designed to be used for single-tube amplification of mRNA using either real-time or end-point amplification strategies.

ArrayScript™ Part nos. AM2048, AM2049	ArrayScript is an M-MLV reverse transcriptase engineered to produce high yields of full-length cDNA. In RNA amplification with less than ~100 ng of total RNA, up to twice as much cRNA is synthesized when ArrayScript is used for the RT step compared to wild type M-MLV, AMV, or other engineered M-MLV reverse transcriptases. ArrayScript is also suitable for other RT and RT-PCR applications where high yields of full-length cDNA are required, such as cDNA library construction, 5' RLM-RACE, etc.
Vortex Adapter Part no. AM10024	The Vortex Adapter attaches to Vortex-Genie® 2 (Scientific Industries) vortex mixers to hold microcentrifuge tube for hands-free agitation.
RNAlater® Solution Part nos. AM7020, AM7021	RNA*later* Tissue Collection: RNA Stabilization Solution is an aqueous sample collection solution that stabilizes and protects cellular RNA in intact, unfrozen tissue and cell samples. RNA*later* Solution eliminates the need to immediately process samples or to freeze samples in liquid nitrogen. Samples can be submerged in RNA*later* Solution for storage at RT, 4°C, or –20°C without jeopardizing the quality or quantity of RNA that can be obtained.

B

Safety

General Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:
Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.).
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of

according to all local, state/provincial, and/or national regulations.

- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/ 29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

- Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

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Documentation and Support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/sds**

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com

At the website, you can:

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

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