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RNaseAlert® Lab Test Kit v2

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Purpose of the product

The RNaseAlert® Lab Test Kit v2 is a convenient and sensitive assay designed to test solutions for the presence of RNase.

RNases are ubiquitous in the environment, and in some biological materials, they are present in relatively high concentrations. RNases also frequently contaminate common molecular biological reagents such as reaction buffers, enzymes such as reverse transcriptase and RNA polymerase, and buffers for RNA purification and storage. Since even minute amounts of ribonuclease (RNase) contamination can ruin experiments involving RNA, it is a good idea to test solutions that will come into contact with RNA with the RNaseAlert® Lab Test Kit v2.

Procedure overview

The procedure is illustrated in the following workflow. Pipet 5 µL of the 10X RNaseAlert® Lab Test Buffer into one of the tubes containing lyophilized RNaseAlert® Substrate v2. Then, add up to 45 µL of the solution to be tested, and incubate for 30 minutes to 1 hour at 37°C. The substrate is a modified RNA oligonucleotide that emits a green fluorescence if it is cleaved by RNase. The fluorescence can be visually detected by short-wave UV illumination or it can be measured in a fluorometer. Solutions with RNase contamination will produce a green glow in the assay, whereas solutions without RNase activity will not fluoresce. The amount of RNase activity is directly proportional to the rate of fluorescence increase. Quantitative measurements can be obtained from a fluorometer.

Resuspend dry RNaseAlert® Substrate v2 with 5 µL of 10X RNaseAlert® Lab Test Buffer

▼

Add up to 45 µL of test sample

▼

Incubate assay 30–60 min at 37°C

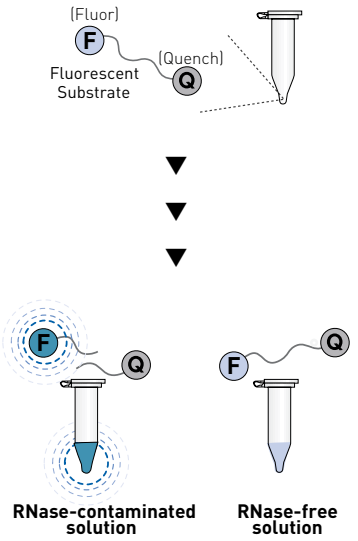
▼

Detect fluorescence

Using a handheld UV light or a transilluminator

or

Using a fluorometer (plate or tube)



Kit contents and storage conditions

Component	Amount	Storage
RNaseAlert® Substrate v2	25 tubes	–20°C
10X RNaseAlert® Lab Test Buffer	250 µL	–20°C
RNase A	500 µL	–20°C
RNase*Zap*® Solution	50 mL	Room temperature
Nuclease-free Water	1.75 mL	Any temperature[1]

[1] –20°C, 4°C, or room temperature

IMPORTANT! Prolonged exposure to light may cause photobleaching of the RNaseAlert® Substrate v2.

Materials required but not provided

Item	Source
Nuclease-free pipettors, tubes, and tips	MLS[1]
Short wave UV light source *or* Fluorometer	MLS

[1] Major laboratory supplier

How to choose which solutions to test

Test solutions at the correct concentration

Because nuclease activity is greatly affected by pH and salt, it is important to test solutions with the exact composition which will be used when RNA is present. For example, contaminating nuclease in one stock solution used to make a reaction buffer may not be active in the assembled reaction. Alternatively, nuclease activity may be detectable in the final mixture, but not in the stock solution used to make it. The RNaseAlert® Lab Test Kit v2 assay is optimized for the detection of RNase A, RNase T1, RNase 1 and micrococcal nuclease; it will also detect other less common nucleases. For example, it can detect Benzonase® nuclease, mung bean nuclease, and S1 nuclease.

Known solution incompatibility

Incompatible solutions	Notes
Gel loading buffers and other darkly colored solutions	Darkly colored solutions may interfere with excitation of the fluorophore or may block its light emission.
Solutions that inhibit RNase activity	The following solutions are known to inhibit RNases: • Solutions with high ionic strength (e.g. 5 M NaCl, 20X SSC, 3 M sodium acetate, etc.) • Solutions with pH <4 or >9 • Chaotropic agents, detergents, chelating agents, or any solutions that denature proteins (e.g. SDS, guanidine thiocyanate, urea, EDTA, etc.)
Solutions that cause chemical instability of the RNaseAlert® Substrate v2	Solutions that chemically degrade the substrate may produce false positive signals. The RNaseAlert® Substrate v2 is unstable in the following types of solutions: • Solutions with pH >9 • Caustic solutions (strong acids and bases, bleach)

Visit the product page at **www.lifetechnologies.com** for a list of commonly used reagents that have been tested with RNaseAlert® technology.

How to determine solution compatibility

1. Test the solution following the procedure in this guide.
2. At the end of the incubation, if no fluorescence above the minus-RNase control is seen, add 5 µL of the supplied RNase A to the completed reaction, and repeat the incubation and signal detection.

   Compatible solutions will strongly fluoresce after incubation with RNase A.

How to test solid surfaces

Pipette tips, pH electrodes, glass beads and other solid surfaces can be tested for RNase by preparing a mock RNaseAlert® reaction as described for the minus-RNase control. Immerse the object in the reaction mixture for a few minutes (pipet up and down for pipette tips), and then check the solution for fluorescence as described in the protocol.

RNaseAlert® Lab Test Kit v2 procedure

Before you begin:

- Clean equipment and plasticware needed with RNase*Zap*® Solution: spray or wipe the surface with a liberal amount of RNase*Zap*® Solution, and rinse twice with high-quality water.
- Decide what kind of plus-RNase control to use (see Table 1).

1. For each solution to be tested, add 5 µL of 10X RNaseAlert® Lab Test Buffer to one tube of RNaseAlert® Substrate v2.

   Prepare one tube for the minus-RNase control.

   Depending on the method chosen for the plus-RNase control (see Table 1), a separate tube may not be needed.

2. Add up to 45 µL of the test solution, and vortex to mix.

Table 1 Sample setup

Sample	Description	
Test sample	45 µL of the test solution. Use the supplied Nuclease-free Water to dilute test solutions to the concentration that will be used in RNA-related experiments.	
Minus-RNase control	Use 45 µL of Nuclease-free Water	
Plus-RNase control	Option 1 (preferred): Add RNase to RNase-free test solutions	After the RNaseAlert® test is finished, add 5 µL of RNase A to a completed reaction for a test solution that showed no detectable RNase, and repeat the incubation and interpretation. This control is preferred, because it validates that a negative result is really negative, not an incompatibility with the RNaseAlert® Lab Test Kit v2).
	Option 2: Conventional plus-RNase control	Use 40 µL of Nuclease-free Water and 5 µL of RNase A.
	Option 3: Add RNase to the minus-RNase control	After the minus-RNase test is finished, and assuming that no RNase was detected, add 5 µL of RNase A to the minus-RNase reaction tube, and repeat the incubation and interpretation.

3. Incubate 30 minutes to 1 hour at 37°C.

   Most contaminated solutions will start to fluoresce after 10 minutes or less, but for optimal sensitivity, the incubation should be continued for 30 minutes to 1 hour.

   (*Optional*) To measure fluorescence with a plate fluorometer capable of real-time measurements, pipet the samples into separate wells of a black 96-well plate, and incubate the plate in the fluorometer, collecting real-time data at 5 minute intervals for 1 hour. If a fluorometer without real-time capabilities will be used, see "How to analyze and interpret results by fluorometer" on page 6. See Figure 1 for example data.

Parameter	Setting
Mode	Kinetic
Excitation/emission (ex/em) maxima	490/520 nm
Gain	Autoscale Alternatively, use medium gain setting initially.
Data collection	Intermittent, 1–1.5 min increments Use intermittent data collection to limit photobleaching.
Temperature	37°C

How to analyze and interpret results by eye

Lay the tubes on a UV transilluminator, turn on the UV light source and inspect for fluorescence.

The spectral output of most UV transilluminators will excite the RNaseAlert® Substrate v2, even though the optimal excitation wavelength is about 490 nm. If you are using a multi-wavelength transilluminator, select the short wavelength setting.

CAUTION! Wear eye and face protection when examining assays with a UV light source.

Sample type	Expected result
Minus-RNase control	There should be minimal or no visible fluorescence. All other samples should be judged by comparison to this control.
Plus-RNase control	The plus-RNase control (regardless of the way it was set up) should fluoresce bright green within 1 hour.
Experimental samples	Samples that are brighter green than the minus-RNase control should be considered contaminated with RNase.

How to analyze and interpret results by fluorometer

RNaseAlert® Lab Test results can be measured in any fluorometer following the manufacturer's instrument guidelines.

- The excitation/emission (ex/em) maxima for the RNaseAlert® Substrate v2 is 490/520 nm. See Figure 1 for example data.
- A medium gain setting is suggested initially.
  Test samples should be measured at the same gain setting as for the minus-RNase control.

Note: If the fluorometer uses a cuvette with a minimum sample volume greater than 50 µL, the reaction can be diluted with Nuclease-free Water up to 2 mL. Dilute the reaction immediately before analysis.

Sample type	Expected result
Minus-RNase control	This sample should have a minimal fluorescence (background). All other samples are judged against this value.
Plus-RNase control	The plus-RNase control should be 20- to 100-fold above the minus-RNase control (background). The fold above background will vary between different fluorometers. Depending on the fluorometer, the fluorescence of the plus-RNase control may exceed the detection range when using the same gain setting as for the minus-RNase control.
Test samples	Solutions which have 2- to 3-fold more fluorescence than the minus-RNase control should be considered contaminated with RNase. Typically, RNase-contaminated solutions fluoresce about 20- to 100-fold more than the minus-RNase control .

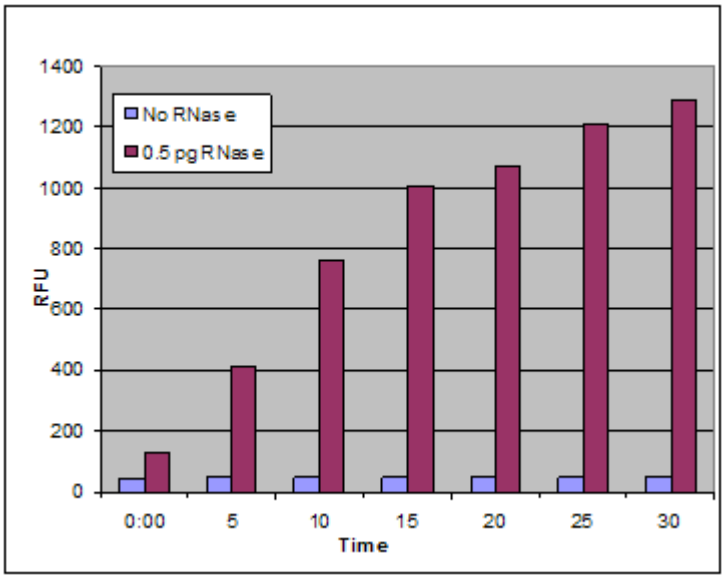


Figure 1 Real-time RNaseAlert® Lab Test Kit v2 data

RNase A at 0.5 pg/well was incubated with the RNaseAlert® Substrate v2 and readings were taken by fluorometer at the indicated time points.

Troubleshooting

Observation	Possible cause	Recommended action
Suspected false positive or false negative results	The solution is not compatible with the RNaseAlert® Lab Test Kit v2 assay. • Solutions which inhibit RNase activity or which block the fluorophore will produce false negative results. • Solutions in which the RNaseAlert® Substrate v2 is unstable may also produce false positive results.	Incompatible solutions cannot be reliably tested with RNaseAlert® Lab Test Kit v2.
Plus-RNase control does not fluoresce after 1-hour incubation	Ambient light is diluting the fluorescence.	Examine the tubes against a dark background, or examine them in a darkroom or dark enclosure.
	The UV light source is not exciting the fluorescent dye.	Darkly colored solutions will block excitation of the fluorophore, and are incompatible with the RNaseAlert® Lab Test Kit v2.
		Verify that the fluorometer is set to excitation/ emission 490/520 nm.
		Try another UV light source. • The light source may not emit the correct wavelength of light. • Some long-wavelength UV light sources are not powerful enough to fully excite the RNaseAlert® Substrate v2.
Minus-RNase control fluoresces	RNase contamination has been introduced. RNase contamination can easily be introduced from the plus-RNase control because it is typically set up at the same time as the experimental samples.	Handle the RNase A supplied with the kit carefully.
		Follow these tips to avoid contaminating the experimental samples and the minus-RNase control. • Assemble the negative control and the experimental samples before pipetting RNase A for the plus-RNase control. • Use nuclease-free pipet tips, and wear fresh gloves to assemble the reactions. • Immediately after pipetting RNase, clean the pipettor with RNase*Zap*® Solution.

Appendix A Safety

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! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/ CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and Support

Obtaining SDSs

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

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

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

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

www.lifetechnologies.com/support

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 (**techsupport@lifetech.com**)
- 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

Limited product warranty

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