USER GUIDE

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RNaseAlert® QC System v2

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Product information

Purpose of the product

The RNaseAlert® QC System 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® QC System v2. Moreover, the RNaseAlert® and DNaseAlert™ QC Systems have been designed to work together seamlessly for simultaneous quantitative detection of RNases *and* DNases in a single sample.

Procedure overview

The procedure is illustrated in the following workflow. Resuspend the lyophilized RNaseAlert® Substrate v2 with 1 mL of the supplied TE Buffer and dispense 10 µL of the solution to each designated sample well of a 96-well plate. Then, add 10 µL of 10X RNaseAlert® Buffer and up to 80 µL of the solution to be tested, and incubate for 30 minutes to 1 hour at 37°C.

The RNaseAlert® Substrate v2 is a modified RNA oligonucleotide that emits a green fluorescence if it is cleaved by RNase. The fluorescence 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. Fluorometers that are capable of real-time or kinetic measurements are particularly useful for monitoring the reaction since the rate of fluorescence increase is proportional to the amount and activity of contaminating RNases.

Resuspend dry RNaseAlert® Substrate v2 with 1 mL of TE Buffer

▼

Dispense 10 µL of RNaseAlert® Substrate v2 per well

▼

Add 10 µL of 10X RNaseAlert® Buffer

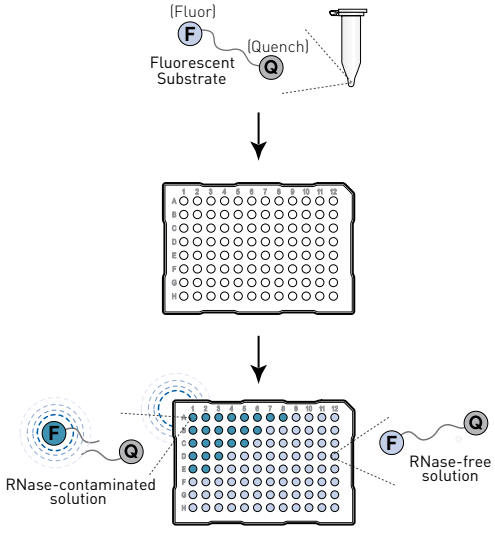
Add up to 80 µL of test solution

▼

Incubate 30–60 min at 37°C

▼

Detect fluorescence in a fluorometer



Kit contents and storage conditions

Component	Amount	Storage
RNaseAlert® Substrate v2	5 tubes	–20°C
10X RNaseAlert® Buffer	5 mL	–20°C
RNase A	500 µL	–20°C
TE Buffer (pH 7.0)	6 mL	–20°C
RNase*Zap*® Solution	250 mL	Room temperature
Nuclease-free Water	50 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 and tips	MLS[1]
Nuclease-free 96-well plates Black plates preferred for lowest background signal	MLS

Item	Source
96-well fluorescence plate reader capable of measuring two or more fluorescent wavelengths in kinetic mode For example: SpectraMax® M5 Multi-Mode Microplate Reader	Molecular Devices

[1] Major laboratory supplier

Methods

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® QC System 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 standard procedure.
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.

Decide on the data collection method

Real-time fluorescence measurements

If you have access to a fluorometer capable of collecting data in real-time, then the RNaseAlert® QC System v2 assay can be evaluated in rigorous kinetic terms. Using real-time data, RNase activities can be compared using enzyme velocity measurements.

Endpoint measurement using a fluorometer

If you do not need real-time data from the RNaseAlert® assay, you can measure the fluorescence on a fluorometer after some defined period of time (for example, 30 minutes). This method is roughly 10 times more sensitive than a visual read-out, and it is quantitative, but it cannot yield the detailed characterization that is possible with steady-state kinetic analyses.

Visual inspection with UV light

The fluorescent glow of the cleaved RNaseAlert® Substrate v2 can be directly visualized by transferring the solution to a thin-wall microcentrifuge tube and shining UV light on it (it is sufficient to simply lay the tubes on a UV transilluminator). RNase contaminated solutions will glow, whereas uncontaminated solutions will not glow. Higher levels of RNase correspond to an increase in the amount of light output. This method is fast and requires no expensive instrumentation, but it is not quantitative.

Standard RNaseAlert® QC System 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 Nuclease-free Water.
- (Optional) If a fluorometer will be used, turn it on and set the following parameters:

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

1. Resuspend 1 tube of RNaseAlert® Substrate v2 with 1 mL TE Buffer, and vortex well to completely dissolve the RNaseAlert® Substrate v2.

   Each screw-top tube contains enough substrate for one 96-well plate.

2. Dispense 10 µL of RNaseAlert® Substrate v2 to the indicated number of wells of a black 96-well plate.

Sample	Number of wells
Minus-RNase control	≥2
Plus-RNase control	≥1
Test samples	2

   Store unused resuspended RNaseAlert® Substrate v2 at –20°C.

3. Add 10 µL of 10X RNaseAlert® Buffer to the wells containing RNaseAlert® Substrate v2.

4. Add 80 µL of each sample and mix.

   If necessary, first bring the volume of the sample to 80 µL with Nuclease-free Water.

Sample	Description	
Test sample (duplicates)	80 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 (duplicates)	80 µL of Nuclease-free Water	
Plus-RNase control	Option 1: Conventional plus-RNase control	75 µL of Nuclease-free Water and 5 µL of RNase A.
	Option 2: Add RNase to RNase-free test solutions	After the assay is complete, add 5 µL of RNase A to a completed reaction for a test solution that showed no detectable RNase, and repeat the incubation. This is an effective control because it confirms that a negative result is not due to an incompatibility with the RNaseAlert® QC System v2.

5. Incubate 30 minutes to 1 hour at 37°C and collect the data.

Data collection method	Procedure
Real-time fluorescence measurements	If you are using a plate fluorometer capable of real-time measurements, incubate the plate in the fluorometer collecting real-time data at 1–1.5 minute intervals for 1 hour using the settings described in this section. If samples are severely contaminated with RNase, a full 1 hour incubation may not be necessary.
Endpoint measurement using a fluorometer	Measure the sample fluorescence after 30–60 minutes using the settings described in this section. **Note:** Highly contaminated samples may be readily detected after just a few minutes.
Visual inspection with UV light	Incubate in a thin-wall microcentrifuge tube for 30–60 minutes 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 30–60 minutes. To read the result, transfer the sample tube to a transilluminator and directly illuminate the sample with long-wave UV light (365 nm). Short-wave UV light (254 nm) can be used if a long-wave light source is not available. The presence of a noticeable glow that is more intense than the minus-RNase control indicates that the sample is contaminated. **Note:** Visual readouts are roughly 10-fold less sensitive than fluorometer measurements.

Interpretation of results

Note: Test samples should be measured at the same gain setting as for the minus-RNase control.

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 .

A

Troubleshooting

Observation	Possible cause	Recommended action
Suspected false positive or false negative results	The solution is not compatible with the RNaseAlert® QC System 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® QC System v2.
Plus-RNase control does not fluoresce after 1-hour incubation	The fluorometer is not exciting the fluorescent dye.	Darkly colored solutions will block excitation of the fluorophore, and are incompatible with the RNaseAlert® QC System v2.
		Verify that the fluorometer is set to excitation/ emission 490/520 nm.
		Try another UV light source. Pipet the plus-RNase control sample into a thin-walled PCR tube and illuminate the tube with long-wave (365 nm) UV light. Shortwave light (254 nm) will also work, but long-wave light is preferred. The liquid should emit a green glow compared to the minus-RNase control sample. If you do see a green glow using this method, then it may be that the light source on your fluorometer does not emit the correct wavelength of light, or it is not powerful enough. If you fail to observe fluorescence in the plus-RNase control sample by simply holding the tube up to a UV light source, repeat the positive control test using 10 µL of RNase A. If the experiment still does not work, contact technical support.

Observation	Possible cause	Recommended action
Minus-RNase control fluoresces	RNase contamination has been introduced.	Handle the RNase A supplied with the kit carefully.
	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.	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 the supplied TE Buffer to resuspend RNaseAlert® Substrate v2. It is certified nuclease-free. • Use nuclease-free pipet tips, and wear fresh gloves to assemble the reactions. • Immediately after pipetting RNase, clean the pipettor with RNase*Zap*® Solution.

B Supplemental procedures

Measure the RNase A detection limit with the RNaseAlert® QC System v2

The following protocol can be used to determine the RNase A detection limits of the RNaseAlert® QC System v2 using any fluorometer. RNase A is widely considered to be the standard for RNase activity.

Note: Use black 96-well plates for best results.

1. Turn on and warm up the fluorometer 96-well plate reader, setting the following parameters:

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

2. Prepare a reaction mix with all the components *except* the RNase A.

Reaction mix (8 samples)	
Component	**Volume**
RNaseAlert® Substrate v2	110 µL
10X RNaseAlert® Buffer	10 µL
Nuclease-free Water	870 µL

3. Prepare 250 µL of 0.1X RNaseAlert® Buffer: mix 2.5 µL of 10X RNaseAlert® Buffer with 247.5 µL of Nuclease-free Water.

4. Set up the 96-well reaction plate as follows:

Sample type	Number of wells	Component	Volume
RNase A dilution	6	Reaction mix	90 µL
Minus-RNase control	2	Reaction mix	90 µL
Assay blank (no substrate)	1	0.1X RNaseAlert® Buffer	100 µL

5. Put the plate in the fluorometer, and collect 5 data points over 5–7.5 minutes.
   Look at a plot of the data to be sure that the points define a straight, horizontal line with minimal scatter. Reading the fluorescence before adding the RNase A serves two purposes: it sets the background level of fluorescence, and it confirms that the buffers and 96-well plate are RNase-free.

6. Prepare a dilution series of RNase A in 0.1X RNaseAlert® Buffer.
   a. Using non-stick tubes, prepare 3 dilutions of RNase A (supplied with the kit at about 1 x 10^{-5} U/µL ±10%) as shown in the table below:

RNase dilution	Dilution instructions	RNase concentration
1:2	15 µL undiluted RNase A + 15 µL 0.1X RNaseAlert® Buffer	5 x 10^{-6} U/µL
1:20	4 µL 1:2 dilution + 36 µL 0.1X RNaseAlert® Buffer	5 x 10^{-7} U/µL
1:200	4 µL 1:20 dilution + 36 µL 0.1X RNase Alert Buffer	5x 10^{-8} U/µL[1]

[1] The detection limit for the RNaseAlert® QC System v2 assay is roughly 5 x 10^{-7} U of RNase A, which is equivalent to about 0.5 pg RNase A.

   These concentrations of active RNase A are at least 500-fold below that of substrate, to enable true steady-state kinetic conditions.

7. Add 10 µL of RNase A dilution or buffer to each substrate-containing well as follows:
   - 10 µL of each dilution of RNase A to duplicate wells (6 wells total)
   - 10 µL of 1X RNaseAlert® Buffer to 2 wells (minus-RNase control, duplicates)

8. Collect fluorometer data points every 1–1.5 minutes over 15 minutes (Figure 1).
   RNase A can adhere to the walls of most 96-well plates (including "low protein binding" plates), thus an initial velocity over the first few minutes of the reaction should be used to accurately calculate the specific activity of the RNase A.

9. Prepare a standard curve of RNase A activity using the initial velocity of the reactions at each RNase dilution.
   Since the RNase A at all concentrations used in the experiment is vastly limiting compared to substrate, the rate of the reaction should be proportional to the amount of input enzyme.
   - Calculate the initial velocity only from the data points from the first 1–3 min after enzyme addition; they represent the rate of substrate turnover when the system is at equilibrium (Figure 2).

- Do not include points that have clearly veered from the initial slope of the reaction rate (i.e., show a reduced slope).

Contaminated samples can then be compared with the standard curve to yield a relative estimate of RNase activity compared to RNase A.

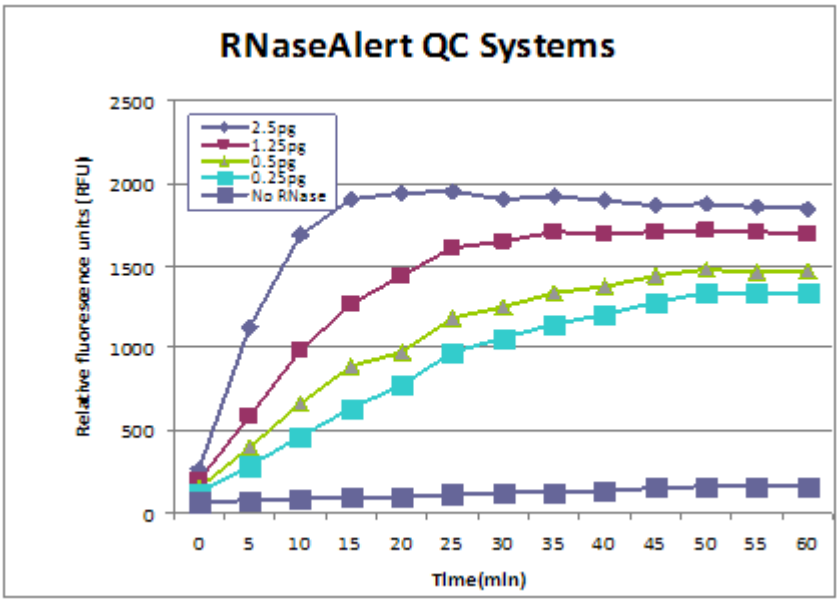


Figure 1 Real-time fluorescent monitoring of RNase A activity

The indicated amounts of RNase A (0–2.5 pg) were monitored in the RNaseAlert® QC System v2 during a 1-hour incubation at 37°C.

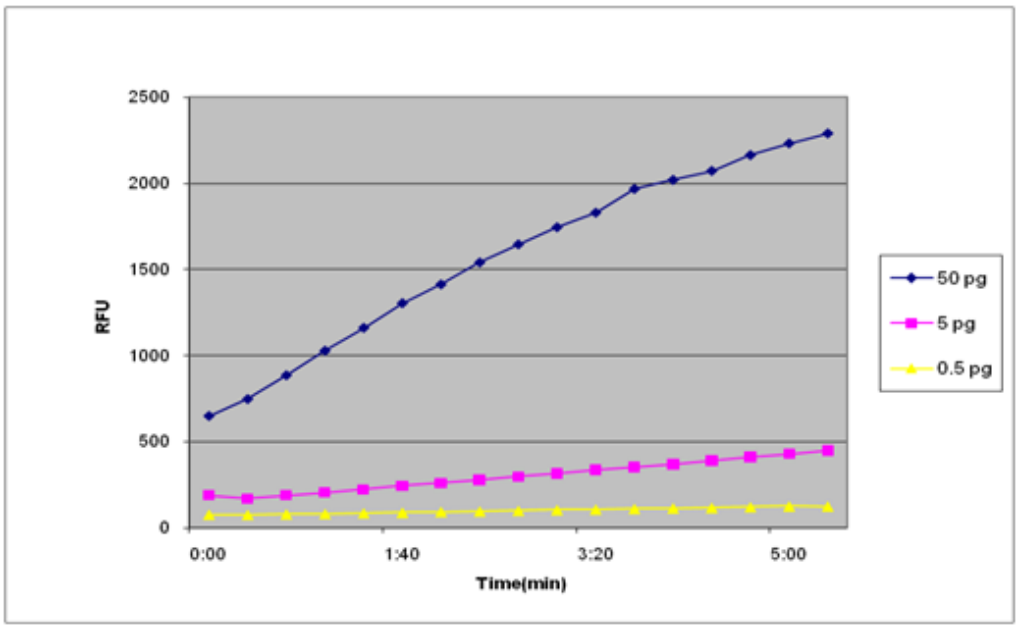


Figure 2 Real-time RNaseAlert® QC System v2 data

10-fold dilutions of RNase A were tested in the RNaseAlert® QC System v2 as described in this section. The plot shows real-time fluorescence data. A plot comparing initial velocities was

linear ($R^2 \geq 0.98$) with RNase A concentration from 0.5–50 pg. Data was monitored on a SpectraMax® M5 Microplate Reader.

Simultaneous measurement of RNases and DNases in a single test sample

The RNaseAlert® QC System v2 was designed to interface seamlessly with the DNaseAlert™ QC System to measure total nuclease activity in a single sample. Since the RNaseAlert® Substrate v2 is spectrally distinct from the DNaseAlert™ Substrate, users can collect kinetic data for both types of nuclease contamination in the same test sample. In-house experiments have demonstrated that the DNase and RNase reactions operate independently in a NucleaseAlert multiplexed reaction. Thus kinetically valid measurements can be obtained for both RNase and DNase reactions simultaneously

Required materials:

- RNaseAlert® QC System v2.
- DNaseAlert™ QC System (Cat. no. AM1970).
- A fluorometer plate reader that can record data from 2 sets of ex/em wavelengths, preferably in a kinetic mode.
- Black 96-well plates preferred, for best results.

1. Turn on and warm up the fluorometer 96-well plate reader, setting the following parameters:

Parameter	Setting
Mode	Kinetic Alternatively, collect endpoint measurements after 1 hour.
DNaseAlert™ excitation/emission (ex/em) maxima	535/556 nm If your fluorometer is filter-based, the most suitable filter is 530/580 nm.
RNaseAlert® 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

2. Resuspend the RNaseAlert® Substrate v2 and DNaseAlert™ Substrate in 1 mL each of TE Buffer, and vortex well to completely dissolve the substrate.

   Use the TE Buffer supplied with each kit.

3. Dispense 10 µL of RNaseAlert® Substrate v2 *and* 10 µL DNaseAlert™ Substrate to each sample well of a black 96-well plate.

Sample	Number of wells
Minus-RNase control	≥2
Plus-RNase control	≥1
Plus-DNase control	≥1
Test samples	2

Store unused resuspended fluorescent substrate at –20°C.

4. Add 10 µL of 10X NucleaseAlert Buffer (supplied in DNaseAlert™ QC System) to each sample well.

5. Add 70 µL of each sample to the appropriate well, and mix.

   If necessary, first bring the volume of the sample to 70 µL with Nuclease-free Water.

Sample	Description	
Test sample (duplicates)	70 µL of the test solution. Use the supplied Nuclease-free Water to dilute test solutions to the concentration that will be used in your experiments.	
Minus-nuclease control (duplicates)	70 µL of Nuclease-free Water	
Plus-RNase control	Option 1: Conventional plus-RNase control	65 µL of Nuclease-free Water and 5 µL of RNase A.
	Option 2: Add RNase to test solution(s) that did not fluoresce	After the assay is complete, add 5 µL of RNase A to a completed reaction for a test solution that did not fluoresce, and repeat the incubation. [1]
Plus-DNase control	Option 1: Conventional plus-DNase control	1. Dilute 1 µL of DNase I in 4 µL 1X NucleaseAlert Buffer to make a 1:5 dilution of DNase I. **Note:** Use 1X NucleaseAlert Buffer made by diluting the 10X NucleaseAlert Buffer supplied with the DNaseAlert™ 1:10 with Nuclease-free Water. 2. Add 65 µL of Nuclease-free Water and 5 µL of the diluted DNase I per well.
	Option 2: Add DNase to test solution(s) that did not fluoresce	After the assay is complete, add 5 µL of a1:5 dilution of DNase I (in 1X NucleaseAlert Buffer) to test samples that did not fluoresce. [1]

[1] This is an effective control because it validates that negative results are not an incompatibility with the assay.

6. Collect data in a fluorometer plate reader over 1 hour.

   An example of the data from such analyses is shown in the following figure.

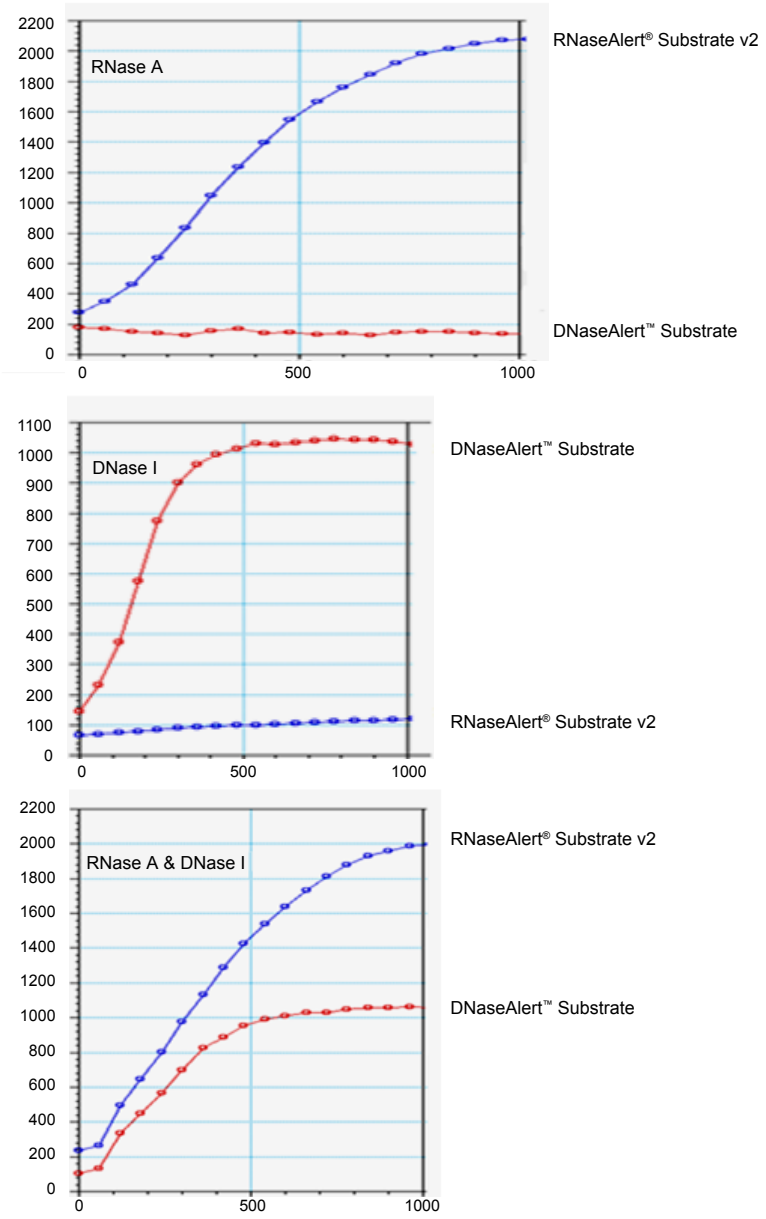


Figure 3 Simultaneous detection of RNases and DNases in real time using both RNaseAlert® QC System v2 and DNaseAlert™ QC System

Either RNase A (5 pg), DNase I (8 ng), or both were added to 100 µL of 1X Nuclease Alert Buffer containing 200 nM RNaseAlert® Substrate v2 and 400 nM DNaseAlert™ Substrate. Reactions were monitored and recorded using a SpectraMAX M5 (Molecular Devices) set to the recommended ex/em wavelengths for the two fluorescent substrates. Note that no change in the DNaseAlert™ Substrate fluorescence is observed when the RNaseAlert® Substrate v2 is cleaved, or vice-versa The lower specific activity of DNase I compared to RNase A is the result of both an intrinsically lower catalytic efficiency by DNase I and the use of the sub-optimal 1X Nuclease Alert Buffer (optimal DNase I activity requires 0.1X Nuclease Alert Buffer).

Interpretation of results

Sample	Expected result and notes
Minus-nuclease control	The minus-nuclease control should have minimal fluorescence (background). All of the experimental and positive control reactions are judged against this value.
Plus-DNase and plus-RNase controls	The plus-DNase and plus-RNase controls should both be 20- to 100-fold above the minus-nuclease control. The fold over background will vary with different fluorometers. **Note:** Depending on the fluorometer, the fluorescence of these controls may exceed the detection range, when using the same gain setting as for the minus-nuclease control.
Test sample	Solutions that have 2- to 3-fold more fluorescence than the minus-RNase or minus-DNase control should be considered contaminated with RNase and DNase, respectively. Samples may have either or both contaminating activities. The emission wavelength of positive signals can be used to distinguish DNase from RNase contamination. The DNaseAlert™ Substrate emits at 556 nm (580 nm for filter-based instruments), whereas the RNaseAlert® Substrate v2 emits at 520 nm.

C

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! 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.

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At the website, you can:

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- 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

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