RNaseAlert® QC System

(Cat #AM1966)

Instruction Manual

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Literature Citation: When describing a procedure for publication using this product, please refer to it as the RNaseAlert® QC System.

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I. Introduction

A. Background

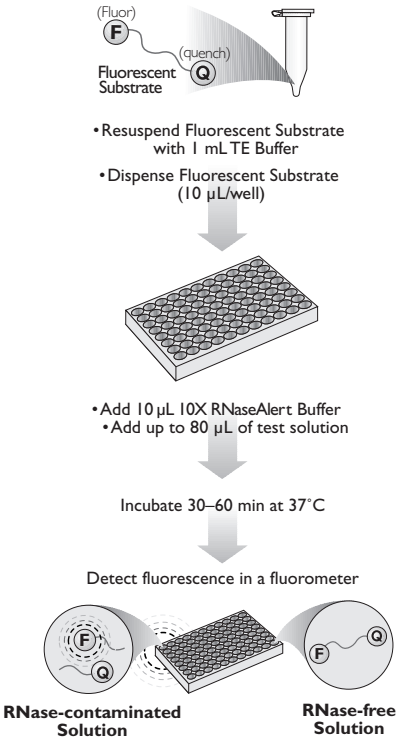
Detect RNases before you start your experiment

Since even minute amounts of ribonuclease (RNase) contamination can ruin experiments involving RNA, it is a good idea to test any solutions that will come into contact with RNA for the presence of RNase. The RNaseAlert® QC System is a convenient and sensitive assay designed for just this purpose. 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. 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.

How the RNaseAlert QC System works

Rapid RNase detection is achieved using a cleavable fluorescent-labeled RNase substrate. The RNaseAlert QC System assay is simple and straightforward; just resuspend the lyophilized RNase Alert Substrate with 1 ml TE and dispense 10 µl of the solution per 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 QC System Substrate 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 RNase Alert Assay since the rate of fluorescence increase is proportional to the amount and activity of contaminating RNases.

Figure 1. RNaseAlert® QC System Procedure



B. Choosing Which Solutions to Test and at What Concentration

Get meaningful results

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 a 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 RNase Alert 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™, mung bean nuclease, and S1 nuclease.

Known solution incompatibility

Most reaction buffers and solutions that are used with RNA can be tested with RNaseAlert QC System. Below are listed the types of solutions that are ***not*** compatible with RNaseAlert QC System.

a. Gel loading buffers and other darkly colored solutions
Darkly colored solutions may interfere with excitation of the fluorophor or may block its light emission, making them incompatible with RNaseAlert QC System.

b. Solutions that inhibit RNase activity
Since RNase must be active for detection, solutions that inhibit RNase activity will not give reliable results with RNaseAlert QC System. 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 a pH <4 or a pH >9
- Chaotropic agents, detergents, chelating agents, or any solutions that denature proteins (e.g. SDS, guanidine thiocyanate, urea, EDTA, etc.)

c. Solutions that cause chemical instability of the RNase Alert Substrate
Solutions that chemically degrade the RNase Alert Substrate are also incompatible for testing with RNaseAlert QC System; they may produce false positive signals. The RNaseAlert QC System Substrate is unstable in the following types of solutions:

- Solutions with a pH >9
- Caustic solutions (strong acids and bases, bleach)

Determining whether a solution can be tested with the RNaseAlert QC System

It is easy to test whether a solution will give valid results in the RNase Alert assay.

First, test the solution following the instructions in section *II.C. Standard RNaseAlert QC System Protocol* starting on page 6. If at the end of the incubation, no fluorescence above the minus-RNase control is seen, add 5 µL RNase A to the completed reaction, and repeat the incubation and signal detection. If the solution can be tested with RNaseAlert QC System, it will strongly fluoresce after incubation with the supplied RNase A.

Testing solid surfaces

Pipette tips, pH electrodes, glass beads and other solid surfaces can be tested for RNase by preparing a mock RNase Alert reaction as described for the minus-RNase control in section II.C. on page 6. 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.

C. Reagents Provided with the Kit and Storage

Amount	Component	Storage
5 tubes	RNaseAlert Substrate	–20°C
5 ml	10X RNase Alert Buffer	–20°C
500 µl	RNase A	–20°C
6 ml	TE Buffer (pH 7.0)	–20°C
250 ml	RNase*Zap*® Solution	room temp
50 ml	Nuclease-free Water	any temp*

* Store Nuclease-free Water at –20°C, 4°C or room temp.

IMPORTANT

Prolonged exposure to light may cause photobleaching of the RNase Alert Substrate.

D. Required Materials Not Provided with the Kit

- Nuclease-free pipettors, and tips
- Nuclease-free 96-well plates (black 96-well plates typically give the lowest background signal, so they are preferred over clear 96-well plates)
- 96-well fluorescence plate reader
  The RNaseAlert QC System Kit was developed using a SPECTRAmax® GEMINI XS 96-well plate fluorometer. Other models, such as Applied Biosystems CytoFluor® Readers, also give excellent results.

E. Related Products Available from Ambion

DNaseAlert™ QC System Cat #AM1970	The DNaseAlert QC System is a fluorometric DNase detection assay designed for high throughput use (96-well plate configuration). This sensitive, easy-to-use system relies on a unique DNA substrate tagged with a fluorescent detector and a dark quencher that emits a bright orange signal when cleaved.
RNase*Zap*® Solution Cat #AM9780, AM9782, AM9784	RNase*Zap* 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 RNase*Zap* Solution.
Buffer Kit Cat #AM9010	RNase-free buffers for critical RNA analysis. These solutions are prepared with high quality reagents, and they are either autoclaved post-packaging, or 0.2 µm filtered before packaging.

II. RNaseAlert® QC System Protocol

A. Options for Data Collection

Data from the RNaseAlert QC System Assay can be obtained in one of three ways. Decide which method will be used before you set up the experiment.

Visual inspection with UV light

The fluorescent glow of the cleaved RNase Alert Substrate can be directly visualized by transferring the solution to a thin-wall microfuge 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.

Endpoint measurement using a fluorometer

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

Real-time fluorescence measurements

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

B. Before You Start

1. Clean equipment and plasticware needed with RNase*Zap*® Solution

Use the RNase*Zap*® Solution (which destroys RNases as well as DNases) to clean pipettors and any plasticware that will be used in the experiment that is suspect with regard to RNases. Simply spray or wipe the surface with a liberal amount of RNase*Zap* Solution, and rinse twice with high-quality water.

2. If a fluorometer will be used, turn it on and set the parameters

Set the following parameters:

- Kinetic mode using a 96-well plate (if available)
- The excitation/emission (ex/em) maxima for the RNase Alert Substrate is 490/520 nm.
- Set the gain to autoscale if possible, alternatively use a medium gain setting initially.

- Collect data at 1–1.5 min increments; intermittent rather than continuous data collection is suggested to limit photobleaching.
- Temperature: 37°C

C. Standard RNaseAlert QC System Protocol

1. Resuspend 1 tube of RNase Alert Substrate with 1 mL TE Buffer

Be sure to vortex well, so that the RNase Alert Substrate is completely dissolved. Each screw-top tube contains enough RNase Alert Substrate for one 96-well plate.

2. Dispense 10 µL of RNase Alert Substrate solution per well of a 96-well plate

Dispense 10 µL of RNase Alert Substrate solution per well into a black 96-well plate for best results. Plan to have at least 2 wells for minus-RNase controls, at least 1 well for a plus-RNase control, and duplicates of each experimental sample.

Unused resuspended RNase Alert Substrate should be stored at –20°C.

3. Add 10 µL of 10X RNase Alert Buffer to each well

Add 10 µL of 10X RNase Alert Buffer only to wells containing RNase Alert Substrate.

4. Add 80 µL of the experimental samples, and mix

To test samples smaller than 80 µL, bring their volume to 80 µL with Nuclease-free Water.

Experimental samples

Typically, 2 repetitions of each experimental sample should be included in the experiment.

The Nuclease-free Water can be used to dilute test solutions to the concentration that will be used in RNA-related experiments.

Minus-RNase control

For the minus-RNase controls, simply add 80 µL Nuclease-free Water instead of sample.

Typically, 2 minus-RNase controls should be included per 96-well plate.

Plus-RNase control

- Add 75 µL of Nuclease-free Water and 5 µL RNase A to a well with RNase Alert Substrate and buffer from step 3.
- Alternatively, you can wait until the assay is complete, and then add 5 µL RNase A to the experimental samples that did not fluoresce. This is an effective control because it validates that a negative result is really negative (and not just an incompatibility with the RNaseAlert QC System).

5. Incubate the reaction 30–60 min at 37°C and collect the data

Real-time fluorescence measurements

If you are using a plate fluorometer capable of real-time measurements, simply incubate the plate in the fluorometer collecting real-time data at 1–1.5 minute intervals for 1 hour using the settings listed in step II.B.2 on page 5. If samples are severely contaminated with RNase, it may be possible to collect the data within a few minutes, and a full 1 hr incubation may not be necessary.

Endpoint measurement using a fluorometer

Measure the sample fluorescence after 30–60 min using the settings listed in step II.B.2 on page 5. Note that highly contaminated samples may be readily detected after just a few minutes.

Visual inspection with UV light

Incubate in a thin-wall microfuge tube for 30–60 min at 37°C

Most contaminated solutions will start to fluoresce after 10 min or less, but for optimal sensitivity, the incubation should be continued for 30–60 min.

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 long-wave light is not available). The presence of a noticeable glow that is more intense than the negative control indicates that the sample is contaminated. Note that visual read-outs are roughly 10-fold less sensitive than fluorometer measurements.

D. Interpretation of Results

Minus-RNase control

This sample will have minimal fluorescence. Any fluorescent signal seen is background. All of the experimental and positive control reactions will be judged against this value.

Plus-RNase control

The plus-RNase control will be 20–100 times above background. The percent above background will vary with different fluorometers.

NOTE

On fluorometers with manual adjustments for gain setting, it may be necessary to use a different gain setting for the plus- and minus-RNase controls to keep them both within the detection range.

Experimental samples

Experimental samples should be measured at the same gain setting as for the minus-RNase control. Solutions which have 2–3 fold more fluorescence than the negative control should be considered RNase contaminated. Typically, RNase contaminated solutions will fluoresce about 20–100-fold more than the minus-RNase control.

III. Troubleshooting

A. Suspected False Positive or False Negative Results

The solution is not compatible with the RNase Alert assay

Solutions that inhibit RNase activity or that block the fluorophor will produce false negative results. Solutions in which the RNaseAlert QC System Substrate is unstable may also produce false positive results. These types of solutions cannot be reliably tested with RNaseAlert QC System (see *Known solution incompatibility* on page 3 and *Determining whether a solution can be tested with the RNaseAlert QC System* on page 3).

B. Plus-RNase Control Does Not Fluoresce After One Hour Incubation

The UV light source is not exciting the fluorescent dye

a. **Darkly colored solutions will block excitation of the fluorophor.**

b. **Verify that the fluorometer is set to excitation/emission: 490/520 nm.**

c. **Try another UV light source.**
You can always directly test for fluorescence by pipetting the plus-RNase control sample into a thin-walled PCR tube and illuminating 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 pink glow compared to the minus-RNase control sample. If you do see a pink glow by eye, then it may be that the light source on your fluorometer does not emit the right wavelength of light or is not powerful enough.

Repeat the plus-RNase control reaction

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 twice the volume (10 µL) of RNase A. If the experiment still does not work, contact Ambion's Technical Services Department (see back cover for contact information).

C. Minus-RNase Control Fluoresces

RNase contamination has been introduced

RNase contamination can easily be introduced from the plus-RNase control because typically it is set up at the same time as the experimental samples. The RNase A supplied with the kit contains a relatively high concentration of RNase and should be handled carefully. The following tips will help to avoid contaminating the experimental samples and the minus-RNase controls:

- Assemble the minus-RNase controls and the experimental samples before pipetting RNase A for the plus-RNase controls.
- Use the supplied TE Buffer to resuspend the RNase Alert Substrate. It is certified nuclease-free.
- Use nuclease-free pipet tips, and wear fresh gloves to assemble RNase Alert experiments.
- Immediately after pipetting RNase, clean the pipettor with RNase*Zap* Solution.

IV. Additional Procedures

A. Measuring the RNase A Detection Limit with RNaseAlert QC System

The following protocol can be used to determine the RNase detection limits of the RNaseAlert QC System using any fluorometer. RNase A is used for the detection limit test because this enzyme is widely considered to be the standard for RNase activity.

1. Turn on and warm up the fluorometer 96-well plate reader

Set the following parameters:

- Kinetic mode using a 96-well plate (if avail)
- The excitation/emission (ex/em) maxima for the RNase Alert Substrate is 490/520 nm.
- Set the gain to autoscale if possible, alternatively use a medium gain setting initially.
- Collect data at 1–1.5 min increments; intermittent rather than continuous data collection is suggested to limit photobleaching.
- Temperature: 37°C

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

There will be 8 samples in the experiment.

Amount	Component
110 µL	RNase Alert Substrate
10 µL	10X RNase Alert Buffer
870 µL	Nuclease-free Water

3. Add 90 µL reaction mix to 8 wells and 90 µL 0.1X RNase Alert Buffer to a 9th well

Use black 96-well plates for best results.

The buffer-only well will serve as the assay blank.

4. Put the plate in the fluorometer, and collect 5 data points over 5–7.5 min

Use the fluorometer settings shown in step 1 on page 10.

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 2 purposes: it sets the background level of fluorescence, and it confirms that the buffers and 96-well plate are RNase-free.

5. Prepare a dilution series of RNase A in 0.1X RNase Alert Buffer

a. First prepare 0.1X RNase Alert Buffer; add 1 µL 10X RNase Alert Buffer to 99 µL Nuclease-free Water.

b. Using non-stick tubes, prepare 3 dilutions of the 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 RNase Alert Buffer	5 x 10^{-6} U/µL
1:20	4 µL 1:2 dilution + 36 µL 0.1X RNase Alert Buffer	5 x 10^{-7} U/µL
1:200	4 µL 1:20 dilution + 36 µL 0.1X RNase Alert Buffer	5 x 10^{-8} U/µL*

* Note that the detection limit for the RNaseAlert QC System 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, i.e., true steady-state kinetic conditions.

6. Add 10 µL of each RNase A dilution to duplicate wells

Start the reaction by adding 10 µL of each dilution of RNase A to duplicate substrate-containing wells (6 wells total). Mix well by pipetting up and down. The remaining 2 substrate-containing wells will be the minus-RNase controls, add 10 µL 1X RNase Alert Buffer to them.

7. Collect fluorometer data points every 1–1.5 min over 15 min

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.

8. Construct a standard curve

The initial velocity is calculated 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. Do not include points that have clearly veered from the ***initial*** slope of the reaction rate (i.e., show a reduced slope). 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. Use the initial velocity of the reactions at each RNase dilution to prepare a standard curve of RNase A activity. Contaminated samples can then be compared with the standard curve to yield a relative estimate of RNase activity compared to RNase A.

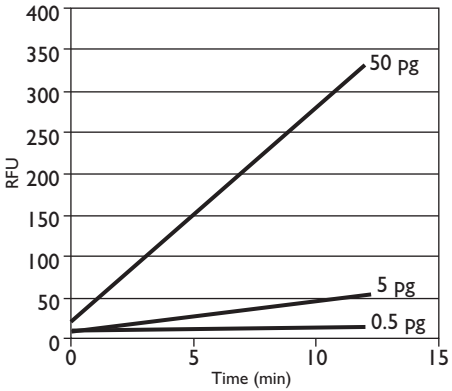


Figure 2. Real-Time RNaseAlert® QC System Data

Plot of RNaseAlert QC System data from an RNase A titration experiment like the one described above. 10-fold dilutions of RNase were tested in the RNaseAlert QC System. A plot comparing initial velocities was linear (R>0.99) with RNase A concentration from 0.5–50 pg. Data were monitored on a SPECTRAmax GEMINI XS microplate spectrofluorometer manufactured by Molecular Devices (Sunnyvale, CA).

B. Simultaneous Measurement of RNases and DNases in a Single Test Sample

Quantitative, real-time kinetic analyses using a 2-color NucleaseAlert method

Many users are concerned about both DNase and RNase contamination. Validation of plastics and solutions as "nuclease-free" requires a sensitive and robust method to detect both RNA and DNA nucleases. The RNaseAlert QC System was designed to interface seamlessly with the DNaseAlert QC System to measure total nuclease activity in a single sample. Since the RNase Alert Substrate is spectrally distinct from the DNaseAlert Substrate, users can collect kinetic data for both types of nuclease contamination in the same test sample. Experiments at Ambion 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.

1. Required materials

This experiment requires the following:

- RNaseAlert Substrate and RNase A from the RNaseAlert QC System (Ambion Cat #AM1966)
- DNaseAlert QC System (Ambion Cat #AM1970)
- A fluorometer plate reader that can record data from 2 sets of ex/em wavelengths preferably in a kinetic mode.

2. Turn on and warm up the fluorometer 96-well plate reader

Set the following parameters:

- Kinetic mode (Use kinetic mode if available, or collect endpoint measurements after 1 hr.)
- DNaseAlert Substrate excitation/emission (ex/em) maxima are 535/556 nm. If your fluorometer is filter-based, the most suitable filter is 530/580 nm.
- RNaseAlert Substrate excitation/emission (ex/em) maxima are 490/520 nm.
- Set the gain to autoscale if possible, alternatively use a medium gain setting initially
- Collect data at 1–1.5 min increments: Intermittent rather than continuous data collection is suggested to limit photobleaching.
- Temperature: 37°C

3. Resuspend the RNase and DNase Alert Substrate(s) in 1 mL TE Buffer

If either of the fluorescent RNase or DNase Alert Substrates are not already resuspended, add 1 mL of the TE Buffer provided with the kit from which the substrate came. Be sure to vortex well, so that the fluorescent substrate is completely dissolved.

4. Dispense 10 µL of each fluorescent substrate solution per well of a 96-well plate

Each well that will be used in the experiment should have:

- 10 µL RNaseAlert Substrate from RNaseAlert QC System
- 10 µL DNaseAlert Substrate from the DNaseAlert QC System

Use black 96-well plates for best results. Plan to have at least 2 wells for minus-nuclease controls, at least 2 wells for plus-nuclease controls (one each for RNase A and DNase I), and duplicates of each experimental sample.

Unused resuspended fluorescent substrate should be stored at –20°C.

5. Add 10 µL of 10X NucleaseAlert Buffer to each well

Add 10 µL 10X NucleaseAlert Buffer only to wells containing the fluorescent substrates.

6. Add 70 µL of the experimental sample, and mix

If you want to test samples smaller than 70 µL, bring the sample volume to 70 µL with Nuclease-free Water.

Experimental samples

Typically, 2 repetitions of each experimental sample should be included in the experiment.

The Nuclease-free Water can be used to dilute test solutions to the concentration that will be used in your experiments.

Minus-nuclease control

For the minus-nuclease controls, simply add 70 µL Nuclease-free Water (instead of sample).

Typically, 2 minus-nuclease controls should be included per 96-well plate.

Plus-DNase control

- Dilute 1 µL of DNase I in 4 µL 1X NucleaseAlert Buffer to make a 1:5 dilution of DNase I. (Be sure to use 1X NucleaseAlert Buffer made by diluting the 10X NucleaseAlert Buffer supplied with the kit 1:10 with Nuclease-free Water).
- Add 65 µL of Nuclease-free Water and 5 µL of the diluted DNase I to a well with the Fluorescent Substrates.
- Alternatively, wait until the assay is complete, then add 5 µL of a 1:5 dilution of DNase I (in 1X NucleaseAlert Buffer) to the experimental samples that did not fluoresce. This is an effective control because it validates that negative results are not simply an incompatibility with the assay.

Plus-RNase control

Add 65 µL of Nuclease-free Water and 5 µL RNase A from the RNaseAlert Kit to a well with the fluorescent substrates. Alternatively, wait until the assay is complete, then add 5 µL of RNase A to the experimental samples that did not fluoresce.

7. Collect data in a fluorometer plate reader over 1 hr

An example of the data from such analyses is shown in Figure 3.

8. Interpretation of results

Minus-nuclease control

This sample will have minimal fluorescence (background). All of the experimental and positive control reactions will be judged against this value.

Plus-DNase and Plus-RNase controls

The fluorescence of these controls may be greater than, or outside the detection range when using the same gain setting as for the minus-DNase control. The plus-DNase and plus-RNase controls will both be 20–100 times above background. The percent above background will vary with different fluorometers.

Experimental samples

Solutions which have 2–3 times more fluorescence than the corresponding 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 RNaseAlert QC System Substrate emits at 556 nm (580 nm for filter-based instruments), whereas the RNaseAlert Substrate emits at 520 nm.

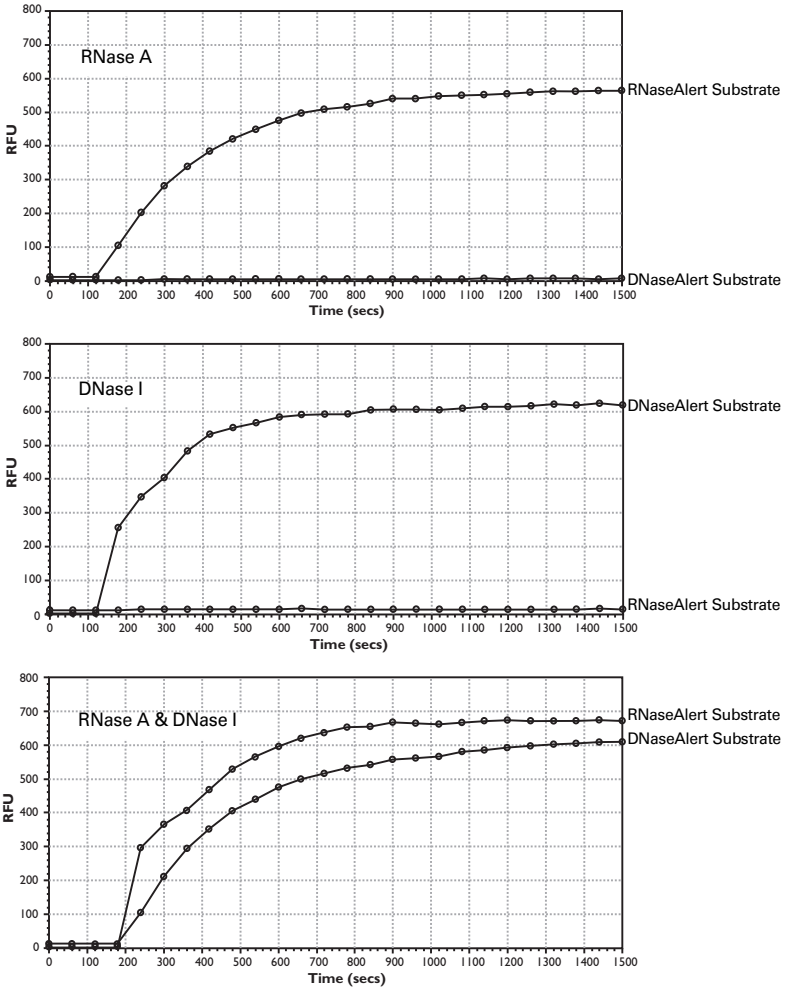


Figure 3. Simultaneous Detection of RNases and DNases in Real-Time Using Both RNaseAlert® and DNaseAlert™

Either RNase A (5 pg), DNase I (8 ng), or both were added to 100 µL 1X NucleaseAlert Buffer containing 200 nM RNaseAlert Substrate and 200 nM DNaseAlert Substrate. Reactions were monitored and recorded using a SpectraMAX Gemini XS (Molecular Dynamics) set to the recommended ex/em wavelengths for the 2 fluorescent substrates. Note that no change in the DNaseAlert Substrate fluorescence is observed when the RNaseAlert Substrate is cleaved, or vice-versa. Moreover, the rate of substrate cleavage using RNase A or DNase I in the multiplexed reaction is within 20% of the rate using either enzyme alone. 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 NucleaseAlert Buffer (optimal DNase I activity requires 0.1X NucleaseAlert Buffer).

V. Appendix

A. Quality Control

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity
Meets or exceeds specification when a sample is incubated with 25 ng labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity
Meets or exceeds specification when a sample is incubated with 300 ng supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity
Meets or exceeds specification when a sample is incubated with 40 ng labeled *Sau*3A fragments of pUC19 and analyzed by PAGE.

B. Safety Information

To obtain Material Safety Data Sheets

- Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address:

  www.ambion.com/techlib/msds

- Alternatively, e-mail your request to MSDS_Inquiry_CCRM@applied-biosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery.
- For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.

- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.