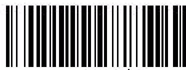
DNaseAlert™ QC System

High Throughput Fluorometric DNase Detection Assay

Part Number AM1970



DNaseAlert™ QC System

(Cat #AM1970)

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I. Introduction

A. Background

Detect DNases before you start your experiment

Like ribonucleases (RNases), deoxyribonucleases (DNases) are ubiquitous enzymes that are present in both the environment and many biological materials. Since DNases degrade DNA, their presence is a threat to many molecular biology experiments. Methods such as PCR rely on the use of plastics, chemicals, and solutions that are free from detectable DNase activity. Published methods for detecting DNase are typically time consuming, not quantitative, and relatively insensitive. In contrast, the DNaseAlert™ QC System is a rapid, user-friendly assay that can detect DNase contamination at minute levels, usually in 10 minutes or less. Moreover, the DNaseAlert and RNaseAlert® QC Systems have been designed to work together seamlessly for simultaneous quantitative detection of RNases ***and*** DNases in a single sample.

How the DNaseAlert QC System works

Rapid DNase detection is achieved using a cleavable fluorescent-labeled DNase substrate. The DNaseAlert QC System assay is simple and straightforward; just resuspend the lyophilized DNase Alert Substrate with TE Buffer and dispense the solution into the wells of a 96-well plate. Add NucleaseAlert Buffer and up to 80 μL of the solution to be tested, and incubate for 10 minutes to 1 hour at 37 °C. The DNase Alert Substrate is a modified DNA oligonucleotide that emits a pink fluorescence when cleaved by DNase. This fluorescence can be readily measured in a fluorometer, or even visualized by eye upon illumination with UV light. Solutions with DNase contamination will produce a pink glow in the assay, whereas solutions without DNase activity will not fluoresce. Fluorometers that are capable of real-time or kinetic measurements are particularly useful for monitoring the DNase Alert Assay since the rate of fluorescence increase is proportional to the amount and activity of contaminating DNases.

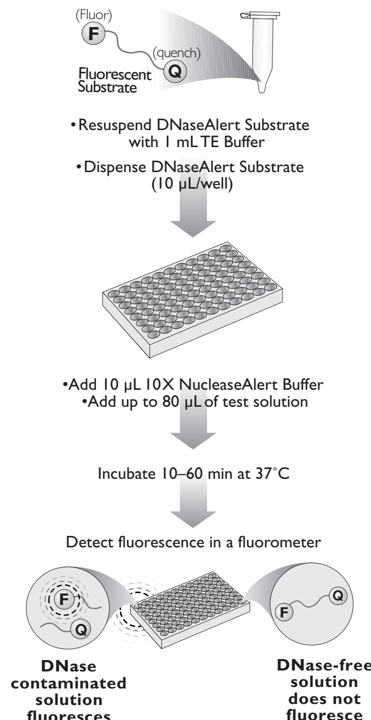

Figure 1. DNaseAlert™ QC System Procedure

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 DNA 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 DNase Alert assay has been standardized to detect DNase I which cleaves double stranded DNA, but it will also detect many other nucleases, even those that cleave single stranded DNA. For example, it can detect Benzonase™, Exonuclease III, mung bean nuclease, micrococcal nuclease, Bal31 nuclease, S1 nuclease, and T7 endonuclease, among others.

Known solution incompatibility

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

b. **Solutions that inhibit DNase activity**
   Since DNase must be active for detection, solutions that inhibit DNase activity will not give reliable results with DNaseAlert QC System. The following solutions are known to inhibit DNases:
   - 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 DNase Alert Substrate**
   Solutions that chemically degrade the DNase Alert Substrate are also incompatible for testing with DNaseAlert QC System; they may produce false positive signals. The DNaseAlert 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 DNaseAlert QC System

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

First, test the solution following the instructions in section II.C. Standard DNaseAlert QC System Protocol starting on page 6. If at the end of the incubation, no fluorescence above the minus-DNase control is seen, add 5 µL of a 1:5 dilution of DNase I to the completed reaction, and repeat the incubation and signal detection. If the solution can be tested with DNaseAlert QC System, it will strongly fluoresce after incubation with the supplied DNase I.

Testing solid surfaces

Pipette tips, pH electrodes, glass beads, and other solid surfaces can be tested for DNase by preparing a mock DNase Alert reaction as described for the minus-DNase 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	DNaseAlert Substrate (2 nmol/tube)	–20 °C
11 mL	10X NucleaseAlert Buffer	–20 °C
20 µL	DNase I (2 U/µL)	–20 °C
6 mL	TE Buffer (pH 7.0)	–20 °C
250 mL	RNase*Zap*® Solution	room temp
50	Non-stick Tubes	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 DNase 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 DNaseAlert 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 Applied Biosystems

Product	Description
RNaseAlert® Lab Test Kit and QC System P/N AM1964, AM1966	The Ambion RNaseAlert products contain a fluorometric RNase detection assay that can detect a wide variety of nucleases. Two formats are available: the RNaseAlert Lab Test Kit for testing of one to several samples, and the RNaseAlert QC System, that provides a 96-well format for high throughput RNase testing.
RNase*Zap*® Solution P/N 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 P/N 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. DNaseAlert™ QC System Protocol

A. Options for Data Collection

Data from the DNaseAlert 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 DNase 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). DNase contaminated solutions will glow, whereas uncontaminated solutions will not glow. Higher levels of DNase 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 DNase Alert assay, you can simply measure the fluorescence on a fluorometer after some defined period of time (e.g. 30 minutes). 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 DNaseAlert QC System assay can be evaluated in rigorous kinetic terms. Using real-time data, DNase 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 DNases. 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 DNase Alert Substrate is 535/556 nm.

If your fluorometer is filter-based, the most suitable filter is 530/580 nm; using this filter will not compromise the sensitivity of DNase I detection.

- Set the gain to autoscale if possible, alternatively use a medium gain setting initially.
- Collect data at 1–1.5 minute increments; intermittent rather than continuous data collection is suggested to limit photobleaching.
- Temperature: 37 °C.

C. Standard DNaseAlert QC System Protocol

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

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

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

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

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

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

Add 10 µL of 10X NucleaseAlert Buffer only to wells containing DNase 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 DNA-related experiments.

Minus-DNase control

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

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

Plus-DNase control

- Prepare ~100 µL of 1X NucleaseAlert Buffer by diluting the 10X solution provided in the kit 1:10 with Nuclease-free Water in a nuclease-free microfuge tube. Store the 1X NucleaseAlert Buffer at –20 °C.

- Dilute 1 µL of DNase I in 4 µL 1X NucleaseAlert Buffer to make a 1:5 dilution of DNase I.
- Add 75 µL of Nuclease-free Water and 5 µL of the diluted DNase I to a well with DNase Alert Substrate and buffer from step 3.
- Alternatively, you can wait until the assay is complete, and 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 a negative result is really negative (and not just an incompatibility with the DNaseAlert QC System).

5. Incubate the reaction for 30–60 minutes 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 DNase, it may be possible to collect the data within a few minutes, and a full 1 hour 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 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 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-DNase 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-DNase control

The plus-DNase 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-DNase 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-DNase control. Solutions which have 2–3 fold more fluorescence than the negative control should be considered DNase contaminated.

III. Troubleshooting

A. Suspected False Positive or False Negative Results

The solution is not compatible with the DNase Alert assay

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

B. Plus-DNase 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: 535/556 nm (or 530/580 nm for filter-based fluorometers).**

c. **Try another UV light source.**
   You can always directly test for fluorescence by pipetting the plus-DNase 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-DNase 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-DNase control reaction

If you fail to observe fluorescence in the plus-DNase 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 a 1:5 dilution of DNase I (diluted in 1X NucleaseAlert Buffer). If the experiment still does not work, contact Technical Services Department (see back cover for contact information).

C. Minus-DNase Control Fluoresces

DNase contamination has been introduced

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

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

D. DNase Alert Signal Plateaus Long Before Substrate is Depleted

DNase I is adhering to the walls of the 96-well plate

We have observed that DNase I can stick to the walls of even "low protein binding" 96-well plates. This effect is probably related to the type of plastic polymer used to the produce the plates, but we often find that the DNaseAlert QC System signal plateaus even when there is still ample substrate that has not been hydrolyzed. This effect is time dependent; the longer the DNase I remains in the plate the greater the level of signal attenuation and the lower the plateau. As a result, this effect is particularly noticeable when low concentrations of DNase I are tested. It is important to note that *stickiness* is an intrinsic property of DNase I that may not be a characteristic of "real-world" DNases. Because DNase I is so sticky, it is very important to measure the ***initial velocity*** of fluorescence (the slope of the line within the first few minutes of the reaction) when quantitating differences in DNase I activity.

IV. Additional Procedures

A. Measuring the DNase I Detection Limit with DNaseAlert QC System

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

NOTE

The detection limit protocol uses 0.1X NucleaseAlert Buffer because it is optimal for DNase I activity. However, we strongly recommend that all experimental solutions be screened using 1X NucleaseAlert Buffer as stated in the protocol. The 1X NucleaseAlert Buffer allows the broadest possible range of nuclease detection and also works well with the RNaseAlert system.

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

Set the following parameters:

- Kinetic mode using a 96-well plate (if available).
- The excitation/emission (ex/em) maxima for the DNase Alert Substrate is 535/556 nm.
  If your fluorometer is filter-based, the most suitable filter is 530/580 nm.
- Set the gain to autoscale if possible, alternatively use a medium gain setting initially.
- Collect data at 1–1.5 minute 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 DNase I

There will be 10 samples in the experiment, the reaction mix below contains 10% overage to compensate for pipetting error.

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

3. Add 90 µL reaction mix to 10 wells and 90 µL 0.1X NucleaseAlert Buffer to an 11th 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 minutes

Use the fluorometer settings shown in step 1 on page 11.

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

5. Prepare a dilution series of DNase I in 0.1X NucleaseAlert Buffer

a. First prepare 0.1X NucleaseAlert Buffer; add 3 µL 10X NucleaseAlert Buffer to 297 µL Nuclease-free Water.
   DNase I activity is reduced by even modest salt concentrations; it is inhibited approximately 2.5-fold in 1X NucleaseAlert Buffer compared to 0.1X NucleaseAlert Buffer. For optimal detection of DNase I, the ionic strength of the reaction buffer must be kept to a minimum. A final concentration of 0.1X NucleaseAlert Buffer strikes a balance between the salt and divalent cation concentration to allow the highest DNase I specific activity.

b. Using the Non-stick Tubes, prepare 4 dilutions of the DNase I (supplied with the kit at 2 U/µL) as shown in the table below:

DNase dilution	Dilution instructions	DNase concentration
1:20	1.25 µL undiluted DNase I + 23.75 µL 0.1X NucleaseAlert Buffer	0.1 U/µL
1:200	2.5 µL 1:20 dilution + 22.5 µL 0.1X NucleaseAlert Buffer	1 x 10^{-2} U/µL
1:2,000	2.5 µL 1:200 dilution + 22.5 µL 0.1X NucleaseAlert Buffer	1 x 10^{-3} U/µL
1:20,000	2.5 µL 1:2,000 dilution + 22.5 µL 0.1X NucleaseAlert Buffer	1 x 10^{-4} U/µL

   These concentrations of active DNase I are at least 500-fold below that of substrate, i.e., true steady-state kinetic conditions.

6. Add 10 µL of each DNase I dilution to duplicate wells

Start the reaction by adding 10 µL of each dilution of DNase I to duplicate substrate-containing wells (8 wells total). Mix well by pipetting up and down. The remaining 2 substrate-containing wells will be the minus-DNase controls, add 10 µL 0.1X NucleaseAlert Buffer to them.

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

DNase I 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 DNase I.

8. Construct a standard curve

The initial velocity is calculated only from the data points from the first 1–3 minutes 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 DNase I 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 DNase dilution to prepare a standard curve of DNase I activity. Contaminated samples can then be compared with the standard curve to yield a relative estimate of DNase activity compared to DNase I.

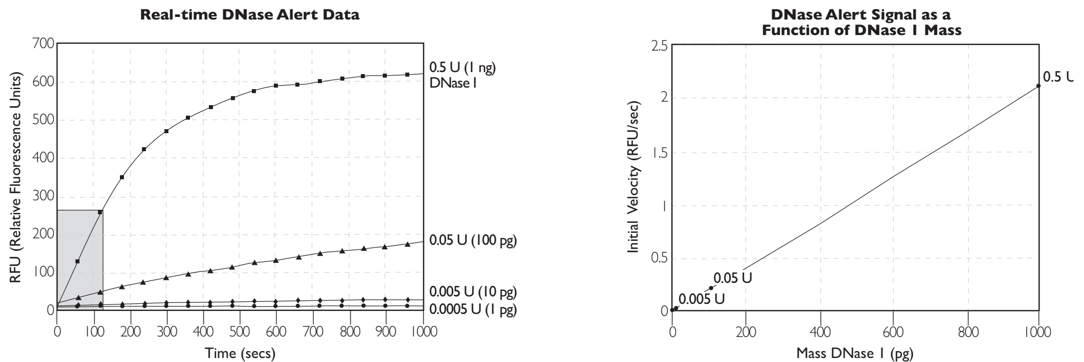

Figure 2. Real-time DNaseAlert QC System Data

Plot of DNaseAlert QC System data from a DNase I titration experiment like the one described above. 10-fold dilutions of DNase were tested in the DNaseAlert QC System. A plot comparing initial velocities was linear (R>0.99) with DNase I concentration from 5×10^{-3} U to 0.5 U (graph on the right). 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 DNaseAlert QC System was designed to interface seamlessly with the RNaseAlert® QC System to measure total nuclease activity in a single sample. Since the DNase Alert Substrate is spectrally distinct from the RNaseAlert 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 (PN #AM1966)
- DNaseAlert QC System (PN #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 hour).
- 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 minute 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 hour

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 DNaseAlert QC System Substrate emits at 556 nm (580 nm for filter-based instruments), whereas the RNaseAlert Substrate emits at 520 nm.

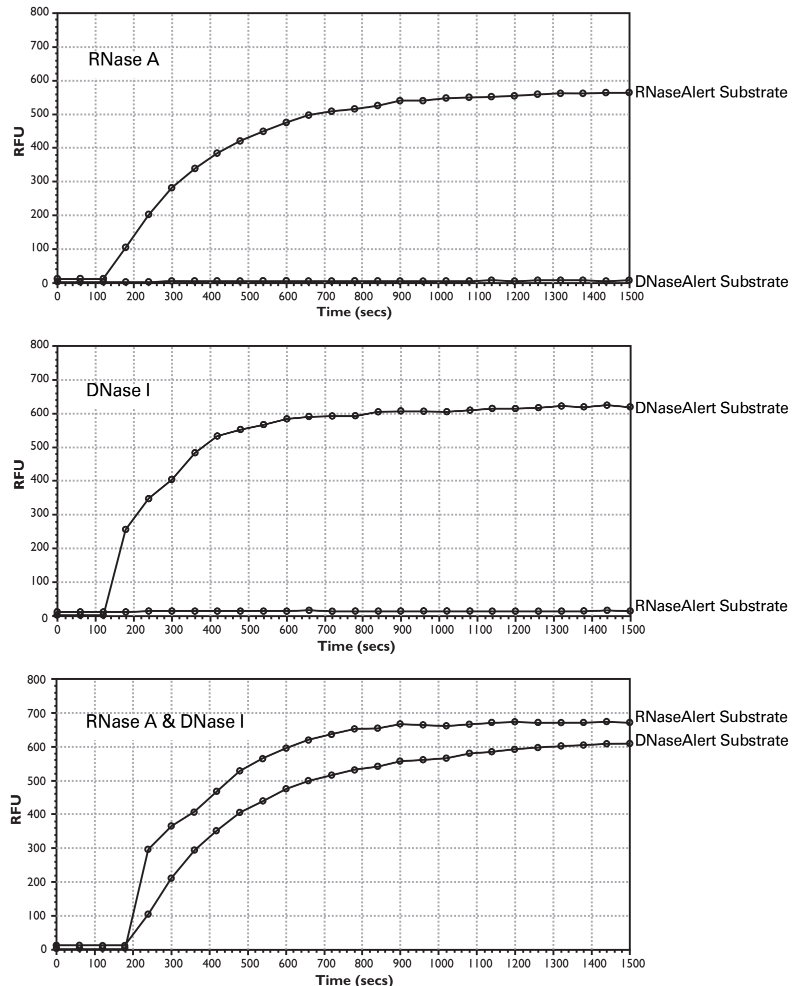

Figure 3. Simultaneous Detection of RNases and DNases in Real-Time Using Both RNaseAlert® and DNaseAlert™

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 labeled RNA and analyzed by PAGE.

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

Exonuclease activity
Meets or exceeds specification when a sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

B. Safety Information

To obtain Safety Data Sheets

- To obtain Safety Data Sheets (SDSs) for any chemical product supplied by Applied Biosystems or Ambion:
- At www.appliedbiosystems.com, select **Support**, then **SDS**. Search by chemical name, product name, product part number, or SDS part number. Right-click to print or download the SDS of interest.
- At www.ambion.com, select **Technical Resources**. Check **SDSs** from the Product Info section, then click **Find Documents**. Select an SDS, then right-click to print or download.
- E-mail (SDS_Inquiry_CCRM@appliedbiosystems.com) or telephone (650-554-2756; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated SDSs unless you request fax or postal delivery. Requests for postal delivery require 1–2 weeks for processing.

For the SDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.

Chemical safety guidelines

To minimize the hazards of chemicals:

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