RNaseAlert® Substrate Nuclease Detection System

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REVISION HISTORY

Version	Release date	Description of changes
3	August 2023	Updated to include recommended incubation time.
2	February 2023	Updated to internal MAPSS standards
1	November 2020	Initital release.

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INTRODUCTION

Nucleases are widely present in the laboratory environment and can interfere with many experiments. In particular, single-stranded RNases are ubiquitous, hard to eliminate, and can rapidly degrade important samples used in microarray studies, real-time PCR, Northern blots, or cDNA cloning. IDT has developed reagents that allow for rapid detection of RNases. These reagents are fluorescence-quenched oligonucleotide probes that start dark but fluoresce after nuclease degradation. The results can be read visually or measured and quantified using fluorometry. RNaseAlert reagents can be used qualitatively or quantitatively to test lab reagents, work surfaces, equipment, and supplies for nuclease contamination.

The RNaseAlert Substrate is a synthetic RNA oligonucleotide that has a fluorescein (R) on one end and a dark quencher (Q) on the other end. Its sequence has been carefully designed to react with a wide variety of ribonucleases. Intact, the substrate has little or no fluorescence. When cleaved by an RNase, the substrate fluoresces green (490 nm or UV excitation, 520 nm emission) and can be detected visually or by using a fluorometer (Figure 1).

Using the RNaseAlert Kit is fast and simple. Lab surfaces or liquid reagents can be tested and demonstrated as "nuclease-free" or "contaminated" in less than an hour. For speed and ease of use, a simple visual assessment can be performed directly at the site of testing. Alternatively, quantitative fluorescent results can be obtained and used to document nuclease testing.

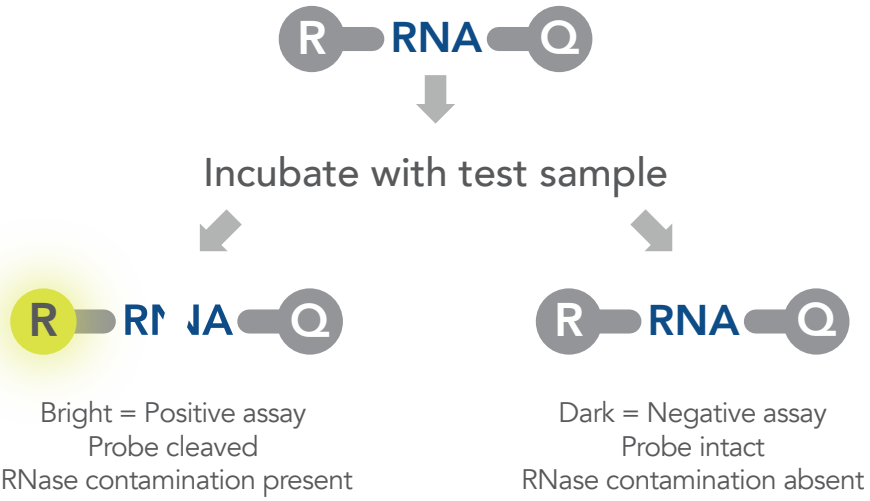

Figure 1. How RNaseAlert Substrate works.

CONSUMABLES AND EQUIPMENT

RNaseAlert Kit contents—Catalog # 11-02-01-02

Component	Quantity	Storage
RNaseAlert Substrate	25 single-use tubes (50 pmol per tube)	–20°C (protect from light to prevent photobleaching)
RNaseAlert Buffer	250 µL	–20°C
Nuclease-Free Water	2 mL	Room temperature
RNase A Enzyme (positive control)	50 µL	–20°C
Nuclease Decontamination Solution	50 mL	Room temperature

Consumables—IDT

Item	Catalog #
RNaseAlert Kit*	11-02-01-02
For liquid solution testing in bulk: • RNaseAlert Substrate, 2 bulk tubes (2nmol per tube)	11-04-02-03

* RNaseAlert Kit components can be purchased separately. Visit the RNaseAlert product page for all ordering options.

Consumables—Other suppliers

Item	Supplier	Catalog #
Nuclease-free tips and pipettes ranging from 1–1000 µL	Various suppliers	Varies
For liquid solution testing in bulk, choose one: • Nuclease-free black, opaque 96-well plate (recommended) • Other nuclease-free 96-well plate or tubes		
Nuclease-free cuvette (optional)		

Equipment

Item	Supplier	Catalog #
Incubator or water bath capable of 37°C incubation (recommended)	Various suppliers	Varies
UV light source for visual detection: • UV transilluminator [300 nm (recommended) or 365 nm)]		
Fluorometer (for quantitative measurement)		

Avoid cross-contamination

Important! The RNaseAlert reagents are supplied free of any contaminating nucleases. It is essential that all subsequent steps be performed under RNase-free conditions.

- Use gloves when handling kit components and performing the DNase detection tests.
- Use nuclease-free pipette tips and tubes.
- If necessary, clean pipettes and other lab surfaces with Nuclease Decontamination Solution before use.

PROTOCOL

Test a liquid solution for RNases with single-use tubes

RNaseAlert Kits contain all reagents needed to perform this method. Prepare one RNaseAlert single-use tube for each sample to be tested. Include 2 additional tubes for the positive and negative controls.

1. Add 5 µL of 10X RNaseAlert Buffer to each RNaseAlert Substrate single-use tube needed for samples and controls.
2. Add 45 µL of sample liquid to each sample tube. Mix well. For both control tubes, add 45 µL of nuclease-free water (provided with kit); also, add 1 µL of RNase A to the positive control tube.

   Note: The final concentration of substrate is 1 µM for quick visual assessment; lower substrate concentrations can be used for fluorometric detection (see Quantitative measurement, below).
3. Incubate at 37°C for 10–60 min.

   Tip: If a temperature-regulated incubator or water bath is not available, incubation can be done at room temperature (2–3X longer incubation time is recommended).
4. Read results visually or with quantitative measurement.
   - **Quick visual assessment:** Place tube on a shortwave (300 nm) UV transilluminator. For best results, use a darkened room. If the tube remains clear, the test is negative and the sample is free of detectable RNase contamination. If the tube glows yellow-green, RNase contamination is present. A longer wavelength (365 nm) UV source can be used, but may not provide the same quality of results.

     Important: Never look directly into a UV light source. Always use protective face shielding.
   - **Quantitative measurement:** The RNaseAlert test is linear over time and can be used for quantitative enzyme activity measurements. Place tube contents in an RNase-free cuvette or microtiter plate and read using a fluorometer on "fluorescein" channel, using 490 nm excitation and 520 nm emission settings. After Step 3 incubation is complete, the sample can be diluted using up to 2 mL nuclease-free water as needed, depending on the size of the detection chamber.

     Caution: If the negative control tube glows, then contamination is present in the tubes, pipette tips, or other piece of lab equipment. Fresh tubes and tips should be obtained and all lab surfaces cleaned with Nuclease Decontamination Solution before proceeding with further tests.
5. Check for potential false negatives. Add 1 µL of RNase A to each negative sample tube. Mix and incubate at 37° C for 10 min.
6. Repeat Step 4 (Read results). All negative tubes should now be positive. Any samples that fail to fluoresce at this point must be considered "failed" and the RNase detection test should be repeated.
   See the Troubleshooting section for help.

Test a solid object or dry surface for RNases with single-use tubes

RNaseAlert Kits contain all reagents needed to perform this method. Prepare one RNaseAlert Substrate single-use tube for each sample to be tested. Include 2 additional tubes for the positive and negative controls.

1. Add 5 µL of 10X RNaseAlert Buffer to each RNaseAlert Substrate single-use tube needed for samples and controls.
2. Add 45 µL of Nuclease-Free Water to each control tube. Add 1 µL of RNase A to the positive control tube only. Mix well.
3. Pipette tips, electrodes, or other small solids can be dipped directly into the prepared reagents. If the object is small enough to be dipped in a tube, add 45 µL of Nuclease-Free Water to each sample tube, mix well, and dip object directly into the prepared reagents. To test surfaces that cannot be dipped into a sample tube:
   - Wipe the surface of interest with a piece of nuclease-free filter paper pre-wetted with Nuclease-Free Water.
   - Soak the filter paper in a small amount of Nuclease-Free Water.
   - Transfer 40 µL of the liquid to a sample tube and mix thoroughly.
4. Incubate at 37 °C for 10–60 min.
5. Read results visually or with quantitative measurement.
   - **Quick visual assessment:** Place tube on a shortwave (300 nm) UV transilluminator. For best results, use a darkened room. If the tube remains clear, the test is negative and the sample is free of detectable RNase contamination. If the tube glows yellow-green, RNase contamination is present. A longer wavelength (365 nm) UV source can be used, but may not provide the same quality of results.

     Caution: Never look directly into a UV light source. Always use protective face shielding.
   - **Quantitative measurement:** The RNaseAlert test is linear over time and can be used for quantitative enzyme activity measurements. Place tube contents in an RNase-free cuvette or microtiter plate and read using a fluorometer on "fluorescein" channel, using 490 nm excitation and 520 nm emission settings. After Step 3 incubation is complete, the sample can be diluted using up to 2 mL nuclease-free water as needed, depending on the size of the detection chamber.

     Note: If the negative control tube glows, then contamination is present in the tubes, pipette tips, or other piece of lab equipment. Fresh tubes and tips should be obtained and all lab surfaces cleaned with Nuclease Decontamination Solution before proceeding with further tests.
6. Check for potential false negatives. Add 1 µL of RNase A to each negative sample tube. Mix and incubate at 37°C for 10 min.
7. Repeat Step 4 (Read results). All negative tubes should now be positive. Any samples that fail to fluoresce at this point must be considered "failed" and the RNase detection test should be repeated. See the Troubleshooting section for help.

Test liquid solutions with RNaseAlert Substrate bulk tubes

Note: In addition to the RNaseAlert Kit contents, this bulk testing method requires RNaseAlert Substrate (2 bulk tubes, 2 nmol per tube; catalog # 11-04-02-03). While bulk substrate can be used in individual tubes, it is more convenient to use microtiter plates. We recommend using opaque black plates that minimize scatter and cross-talk between wells.

Important: Always include negative and positive control wells. Use duplicate or triplicate sample wells if quantitative results are desired.

1. Rehydrate the bulk RNaseAlert Substrate by adding 1 mL Nuclease-free water to the tube and mixing well. Final substrate concentration is 2 µM (20 pmol substrate in 10 µL).
2. Add 10 µL of RNaseAlert Substrate to each sample and control well.
3. Add 10 µL of 10X RNaseAlert Buffer to each sample and control well.
4. Add 80 µL of sample to the sample wells. Add 80 µL of Nuclease-Free Water to each control well. Add 1 µL of RNase A to the positive control well. Mix thoroughly.

   Note: The recommended final concentration of substrate is 200 nM for a standard 96-well fluorometer; more dilute solutions can be used with certain types of cuvette or fluorometer.
5. Incubate at 37°C for 30–60 min.
6. Read plate in a fluorometer using fluorescein channel (490 nm excitation, 520 nm emission). Results can be read as a simple end-point or can be examined in real time to obtain quantitative kinetic curves.
7. Check for potential false negatives by adding 1 µL of RNase A to each negative sample well. Mix and incubate at 37°C for 10 min.
8. Re-examine for fluorescence. Any samples that fail to glow during this step may be missing substrate or contain a factor that inhibits RNase activity. See the Troubleshooting section for help.

TROUBLESHOOTING

Issue	Possible cause	Suggested solution
False negatives	**Presence of RNase inhibitors** Solutions with extreme pH, strong ionic strength, or detergents can block RNase action, preventing detection of contaminants that are present.	Test your solution for inhibitors. • Set up a single-use tube with your test solution and add 1 µL RNase A. • If the tube does not convert to "positive" after a 1-hour incubation, your test solution is incompatible with the RNaseAlert System.
	Low pH solutions Solutions with pH <7.0 will decrease the efficiency of fluorescein fluorescence.	If possible, adjust the pH of the test solution to obtain pH >7.0 and <9.0.
	Substrate loss 1. The RNaseAlert Substrate is provided dried down. The dry pellet can dislodge from the bottom of the tube and may be lost from the tube when opened; dry oligos can be electrostatically attracted to laboratory gloves. 2. Prolonged exposure of the RNaseAlert Substrate to light can lead to photobleaching of the fluorescent dye and decrease test reliability.	1. a. Spin down tubes before opening. b. Perform a positive control test. Always perform a positive control test on single-use tubes with negative results. A positive result in another tube, even from the same kit, is not sufficient. 2. Store bulk substrate and single-use tubes in the dark.

Issue	Possible cause	Suggested solution
False positives	**Contamination** Nuclease contamination of tubes, pipette tips, and other lab equipment can lead to false positives.	Perform a negative control test. • A negative control must be included. Fluorescence in the negative control tube indicates contamination in the experimental setup Obtain new supplies and clean work area. • If contamination is suspected, obtain fresh tubes and pipette tips and clean all lab surfaces with Nuclease Decontamination Solution before repeating the test.
	Quencher exhaustion Prolonged exposure of the substrate to UV light can damage the quencher. A tube left on an intense shortwave (254 nm) UV source can turn "positive" even when no nuclease is present.	Read your visual results immediately to limit exposure to UV light.
	Substrate degradation The RNaseAlert Substrate contains RNA bases and can be degraded by non-enzymatic methods.	Avoid contact with alkaline solutions (pH >9.0) or temperatures >70°C.
	Substrate stimulation Certain organic solvents will disrupt quenching. In particular, the RNaseAlert Substrate always glows in acetonitrile.	Avoid use of solutions containing acetonitrile.

RNaseAlert Substrate Nuclease Detection System

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