RNaseAlert™ Substrate Nuclease Detection System

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, sensitive detection of RNases. These reagents are fluorescence-quenched oligonucleotide probes that start dark but fluoresce after nuclease degradation. The assay can be read visually or measured and quantified using fluorometry. Assays can be used qualitatively or quantitatively to test lab reagents, work surfaces, equipment, and supplies for nuclease contamination. The reagents can also be used as highly sensitive substrates for enzyme kinetic studies.

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 optimized 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).

Use of the RNaseAlert Kit is fast and simple. Lab surfaces or liquid reagents can be tested and verified as "nuclease-free" or "contaminated" in less than an hour. For speed and ease of use, a simple visual assay can be performed directly at the site of testing.

Alternatively, quantitative fluorescent results can be obtained and used to document nuclease testing for GMP or manufacturing needs.

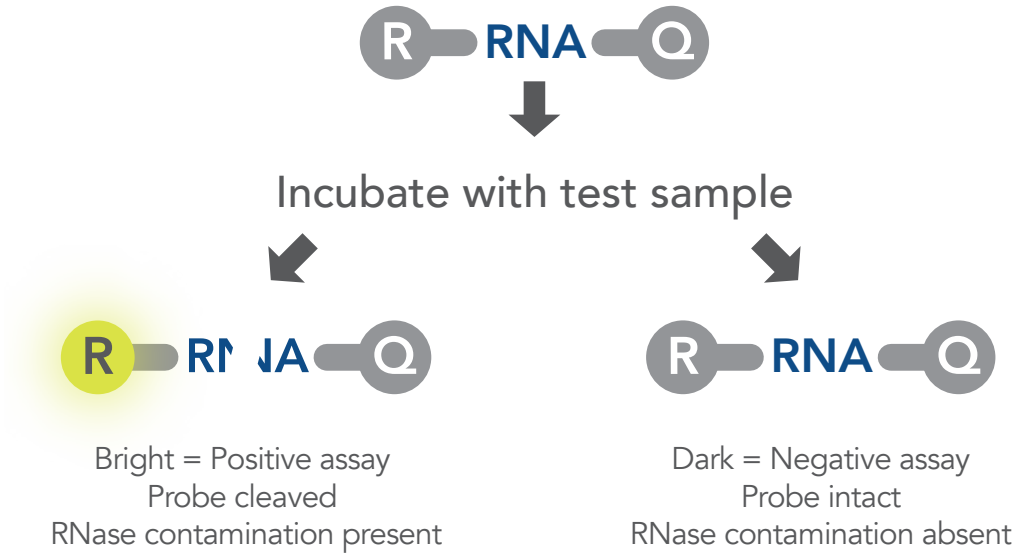

Figure 1. Nuclease detection by RNaseAlert Substrate.

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RNaseAlert Kit components

- 25 single-use tubes RNaseAlert Substrate, 50 pmol per tube
- 250 µL RNaseAlert Buffer
- 2 mL Nuclease-Free Water
- 50 µL RNase A (positive control)
- 50 mL Nuclease Decontamination Solution

Reagent quality is guaranteed for six months from date received when stored at –20°C.

Nuclease Decontamination Solution should be stored at room temperature.

RNaseAlert Substrate

- 25 single-use tubes, 50 pmol per tube

  or

- 2 tubes of bulk substrate, 2 nmol per tube (100 assays per tube)

Note: RNaseAlert Substrate should be stored long term at –20°C in the dark to prevent photobleaching.

RNaseAlert PROTOCOL

Before starting

The RNaseAlert Substrate and Buffer are supplied free of any contaminating nucleases. It is essential that all subsequent steps be performed under RNase-free conditions.

- Use gloves at all times when handling kit components and performing the assay.
- Use RNase-free pipette tips and tubes.
- If necessary, clean pipettors and other lab surfaces with Nuclease Decontamination Solution before use.

Assay setup

Single-use tubes, testing a liquid solution

1. **Add 5 µL of 10X RNaseAlert Buffer to an RNaseAlert Substrate tube.** Use 1 tube of RNaseAlert substrate for each reagent tested. Include 2 control tubes. Thus, if testing 8 samples, you will set up 10 tubes (8 unknowns plus 1 negative control and 1 positive control).
2. **Add 45 µL test sample to the tube. Mix well. For both control tubes, add 45 µL of RNase-free water (provided with kit); also, add 1 µL of RNase A to the positive control tube.** The final concentration of substrate is 1 µM for the visual assay; lower substrate concentrations can be used with fluorometric detection (see below).
3. **Incubate 10 minutes to 1 hour at 37°C.** Greater sensitivity is achieved with longer incubations. 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 to achieve similar sensitivity).
4. **Quick visual confirmation of results:** Place tube on a shortwave (300 nm) UV transilluminator. If the tube remains clear, the assay 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 sensitivity will be lower.

   Important! Never look directly into a UV light source. Always use protective eye shielding.

   Best results will be obtained using the visual assay format if done in a darkened room.

   Quantitative results: The RNaseAlert assay 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 RNase-free water as needed, depending on the size of the detection chamber.

   Assessing controls: If the negative "control" tube (RNase-free water test) 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 assays.
5. **Validate all "negative" assays by adding 1 µL of RNase A (provided in kit) to each tube. Mix and incubate for 10 minutes at 37°C. Repeat detection procedure as before.** All negative tubes should now be positive. Any tube that fails to fluoresce at this point must be considered a "failed" assay and should be repeated. See troubleshooting guide at the end of this manual for help with assay failures.

Assay setup

Single-use tubes, testing a dry surface

Lab surfaces can be assayed for RNase contamination using RNaseAlert. Pipette tips, electrodes, or other small solids can be directly dipped into an assay set up like a control reaction (substrate + buffer + water). Following incubation, the presence or absence of a fluorescent signal will indicate the presence or absence of nuclease contamination. If contamination is detected, the surface can be decontaminated with Nuclease Decontamination Solution.

For testing surfaces that cannot be dipped into an assay tube, sample the surface using the following method. Wipe the surface of interest using a piece of RNase-free filter paper. Pre-wetting the filter paper with RNase-free water will improve sensitivity. After wiping down the surface of interest, soak the filter paper in a small amount of RNase-free water. Transfer the liquid to an assay tube and proceed as above for testing liquid samples.

Assay setup

Bulk substrate, testing a liquid solution

1. **Rehydrate the bulk RNaseAlert Substrate by adding 1 mL RNase-free water to the tube and mixing well.** This will result in a substrate concentration of 2 µM, or 20 pmol substrate in 10 µL. While bulk substrate can be used in individual tubes, it is most convenient to use in microtiter plates. IDT recommends use of opaque black plates that minimize scatter and cross-talk between wells.
2. **Add 10 µL of RNaseAlert Substrate to each well.** Always include negative and positive control wells; use duplicate or triplicate sample wells if quantitative results are desired.
3. **Add 10 µL of 10X RNaseAlert Buffer to each well. Add 80 µL sample to each well. Mix and incubate for 30–60 minutes at 37°C.** Recommended final concentration of substrate is 200 nM for a standard 96-well fluorometer; more dilute solutions can be used with a more sensitive cuvette or fluorometer.
4. **Read plate in a fluorometer using fluorescein channel (490 nm excitation, 520 nm emission).** Assay can be read as a simple end-point assay or can be examined in real time to obtain quantitative kinetic curves.
5. **Validate all "negative" assays by adding 1 µL stock RNase A to each well. Mix and incubate at 37°C for 10 minutes.** Re-examine for fluorescence; all wells should now be maximally positive. Any wells that are negative will either be missing substrate or contain a factor that inhibits RNase activity.

TROUBLESHOOTING GUIDE

False negatives

RNase inhibitors

Solutions with extreme pH, strong ionic strength, or detergents can block RNase action, preventing detection of contaminants that are present. It is simple to test if your solution contains a component that inhibits the RNaseAlert assay. Set up a standard assay with your test solution and add 1 µL control RNase A solution. If the assay does not convert to "positive" after a one-hour incubation, then your test solution is incompatible with the RNaseAlert System.

Low pH solutions

Solutions with pH < 7.0 will decrease the efficiency of fluorescein fluorescence, lowering assay sensitivity.

Substrate loss

The RNaseAlert Substrate is provided lyophilized. 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. It is essential to perform a positive control as recommended in the assay protocol. For single-use tubes, each tube should receive positive control testing to confirm validity of a negative result. Positive control testing done on other tubes, even from the same kit, is not sufficient.

Prolonged exposure of the RNaseAlert Substrate to light can photobleach the fluorescent dye and decrease assay sensitivity. Store bulk substrate and assay tubes in the dark.

False positives

Contamination

Nuclease contamination of tubes, pipette tips, and other lab equipment can lead to false positives. A negative control tube must be included with each set of assays performed.

Quencher exhaustion

Prolonged exposure of the substrate to UV light can damage the quencher. A visual assay left on an intense short-wave (254 nm) UV source can turn positive even when no nuclease is present.

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.

RNaseAlert Substrate Nuclease Detection System

Technical support: applicationsupport@idtdna.com

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