RNaseAlert® Lab Test Kit

Fluorometric RNase Detection Assay
Part Number AM1964

Applied Biosystems

A. Background

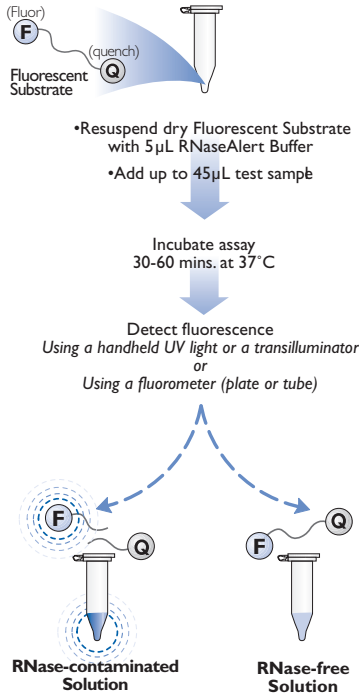
Detect RNase before you start your experiment

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. RNaseAlert® Lab Test Kit 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.

How RNaseAlert Lab Test works

Rapid RNase detection is achieved using a cleavable fluorescent-labeled RNase substrate developed as a collaborative effort by Ambion and Integrated DNA Technologies, Inc. (patent pending). The RNaseAlert Lab Test assay is simple and straightforward; just pipet 5 µL of the 10X RNaseAlert Lab Test Buffer into one of the tubes containing lyophilized Fluorescent Substrate. Then, add up to 45 µL of the solution to be tested, and incubate for 30 min to 1 hr at 37°C. The Fluorescent Substrate is a modified RNA oligonucleotide that emits a green fluorescence if it is cleaved by RNase, the fluorescence can be visually detected by short-wave UV illumination or measured in a fluorometer. Solutions with RNase contamination will produce a green glow in the assay, whereas solutions without RNase activity will not fluoresce. The amount of

RNase activity will be directly proportional to the rate of fluorescence increase. Quantitative measurements can be obtained from a fluorometer.



B. Kit Components & Storage Conditions

Amount	Component	Storage
25 tubes	Fluorescent Substrate	–20°C
250 µL	10X RNaseAlert Lab Test Buffer	–20°C
500 µL	RNase A	–20°C
50 mL	RNase*Zap*®	room temp
1.75 mL	Nuclease-free Water	any temp*

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

IMPORTANT

Prolonged exposure to light will cause photobleaching of the Fluorescent Substrate.

Components/Equipment not provided with kit

Nuclease-free pipettors, tubes, and tips

Short wave UV light source, or a Fluorometer

C. Related Products from Applied Biosystems

RNase*Zap*®

P/N AM9780, AM9782, AM9784

RNase Decontamination Solution. RNase*Zap* is simply sprayed or poured onto surfaces to instantly inactivate RNases. Rinsing twice with water will eliminate all traces of RNase and RNase*Zap*.

Buffer Kit

See web or print catalog for P/Ns

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.

SUPERase•In™

P/N AM2694, AM2696

SUPERase•In is the most effective RNase inhibitor available. It can be used wherever traditional RNase inhibitor is used, but unlike traditional RNase inhibitor, it doesn't require DTT, and it remains active in a much wider variety of reaction conditions. SUPERase•In also protects RNA from more RNases and at higher concentrations than RNase inhibitor.

D. Choosing Which Solutions to Test

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 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 Lab Test

assay is optimized for the detection of RNase A, RNase T1, RNase 1 and micrococcal nuclease; it will also detect other less common nucleases.

Known solution compatibility

Most reaction buffers and solutions that are used with RNA can be tested with RNaseAlert Lab Test. Note that gel loading buffers and other darkly colored solutions that interfere with excitation of the fluorophor or which block its light emission cannot be tested with RNaseAlert Lab Test.

In addition, solutions that inhibit RNase activity will not give reliable results with RNaseAlert Lab Test because in this assay, the RNase must be active to be detected. The following solutions are known to inhibit RNases:

- Solutions with high ionic strength
- Solutions with a pH <4 or >9

Finally, solutions which cause chemical instability of the Fluorescent Substrate are also incompatible for testing with RNaseAlert Lab Test; they may produce false positive signals. The Fluorescent Substrate is unstable in the following types of solutions:

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

A table listing many compounds that have been tested in RNaseAlert Lab Test can be found at www.ambion.com.

Determining whether a solution can be tested with the RNaseAlert Lab Test Assay

Fortunately, it is easy to test whether a solution will give valid results in the RNaseAlert Lab Test assay. Simply test the solution following the procedure in this booklet. 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 detection of fluorescence. If the solution can be tested with RNaseAlert Lab Test, it will 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 RNaseAlert Lab Test reaction as described for the minus-RNase control on

page 5. Immerse the object in the reaction mixture for a few min (pipet up and down for pipette tips), and then check the solution for fluorescence as described in the procedure.

E. RNaseAlert® Lab Test Kit Procedure

1. Before you start

Use the RNase*Zap* to clean pipettors, and any plasticware that is suspect with regard to RNases. Simply spray or wipe the surface with a liberal amount of RNase*Zap*, and rinse twice with high-quality water.

2. Add 5 µL of 10X RNaseAlert Lab Test Buffer to one of the tubes of Fluorescent Substrate

Use 1 tube of Fluorescent Substrate for each solution to be tested, and prepare a tube for the minus-RNase control. There are a few options for the plus-RNase control, so a separate tube may not be needed depending on the method used (see *Plus-RNase control* on page 5).

3. Add up to 45 µL of the test solution, and vortex to mix (bring the sample volume to 45 µL with Nuclease-free Water if necessary)

Experimental samples

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 control, simply add 45 µL Nuclease-free Water (instead of sample).

Plus-RNase control

The Plus-RNase control can be run in three different formats; methods a and c (below) conserve Fluorescent Substrate, but they cannot be read at the same time as the experimental samples, because they are done after the experiment by re-using either the minus-RNase control, or one of the experimental samples that tested negative for RNase. Be very careful to avoid RNase A carry-over to sample tubes; this will cause false positive results.

a. Add RNase to RNase-free test solutions

Any test solution that showed no detectable RNase can be used as a plus-RNase control. After the experiment is finished add 5 µL RNase A to the completed reaction, and repeat the incubation and interpretation.

This control is preferred, because it validates that a negative result is really negative (and not just an incompatibility with the RNaseAlert Lab Test system).

b. Conventional plus-RNase control

Add 40 µL of RNase-free Water and 5 µL RNase A to a tube with Fluorescent Substrate and RNaseAlert Lab Test Buffer.

c. Add RNase to the minus-RNase control

After the minus-RNase control experiment is finished, and assuming that no RNase was detected, add 5 µL RNase A to the minus-RNase reaction tube, and repeat the incubation and interpretation.

4. Incubate 30 min to 1 hr 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 min to 1 hr.

If you have access to a plate fluorometer capable of real-time measurements, simply pipet your samples into separate wells of a 96-well plate, and incubate the plate in the fluorometer collecting real-time data at 5 min intervals for 1 hr (a medium gain setting is recommended initially). Intermittent data collection is suggested to limit photobleaching. The excitation/emission (ex/em) maxima for the Fluorescent Substrate is 490/520 nm, and a medium gain setting is suggested initially. (If a fluorometer without real-time capabilities will be used, see section G on page 7.)

F. Interpretation of Results by Eye

Lay the tubes on a UV transilluminator, turn on the UV light source and inspect for fluorescence. Be sure to wear eye and face protection when examining assays with a UV light source.

The spectral output of most UV transilluminators will excite the Fluorescent Substrate, even though the optimal excitation wavelength is about 490 nm. If you are using a multi-wavelength transilluminator, the short wavelength setting should be selected.

Minus-RNase control

There should be minimal or no visible fluorescence from the minus-RNase control. All other samples should be judged by this standard.

Plus-RNase control

The plus-RNase control (regardless of the way it was set up) should fluoresce bright green within 1 hr.

Experimental samples

Samples that fluoresce bright green have RNase activity. Also, samples that are more green than the negative control contain RNase, and should not be used in experiments with RNA.

G. Interpretation of Results by Fluorometer

RNaseAlert Lab Test results can be measured in any fluorometer following the manufacturer's instrument guidelines. The excitation/emission (ex/em) maxima for the Fluorescent Substrate is 490/520 nm, and a medium gain setting is suggested initially.

NOTE

If the reaction will be analyzed in a fluorometer that uses a cuvette with a minimum sample volume greater than 50 µL, then the reaction can be diluted with Nuclease-free Water up to 2 mL. This should be done immediately before analysis.

Minus-RNase control

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

Plus-RNase control

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

Experimental samples

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

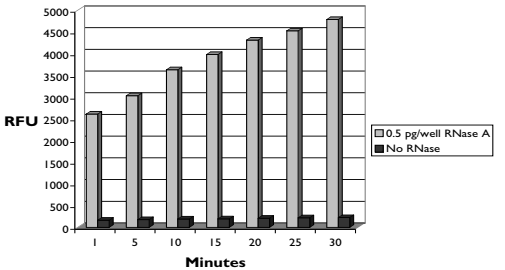


Table 1. Real-time RNaseAlert® Lab Test Kit Data

Comparison of RNaseAlert Lab Test results from wells containing either nuclease-free TE Buffer, or TE containing 0.5 pg/100 µL RNase A. Data were monitored on a SPECTRAmax® GEMINI XS Microplate Spectrofluorometer manufactured by Molecular Devices (Sunnyvale, CA).

H. Troubleshooting

1. Suspected false positive or false negative results

The solution is not compatible with the RNaseAlert Lab Test assay

Solutions which inhibit RNase activity or which block the fluorophor will produce false negative results. Solutions in which the Fluorescent Substrate is unstable may also produce false

positive results. These types of solutions cannot be reliably tested with RNaseAlert Lab Test (see "Known solution compatibility" on page 4).

2. Plus-RNase control does not fluoresce after 1 hr incubation

Ambient light is diluting the fluorescence

- It helps to examine the tubes against a dark background, or to examine them in a darkroom or dark enclosure.

The UV light source is not exciting the fluorescent dye

- Darkly colored solutions will block excitation of the fluorophor.
- Verify that the fluorometer is set to excitation/emission: 490/520 nm.
- Try another UV light source.
  If there is no fluorescence from the plus-RNase control, the light source may not emit the right wavelength of light or it may not be powerful enough. Some long wavelength UV light sources are not powerful enough to fully excite the Fluorescent Substrate.

3. 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 high concentration of RNase and should be handled carefully. The following tips will help 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 the RNaseAlert Lab Test fluorescent substrate. It is certified nuclease-free.
- Use nuclease-free pipet tips, and wear fresh gloves to assemble RNaseAlert Lab Test experiments.
- Immediately after pipetting RNase, clean the pipettor with RNase*Zap*.

I. Quality Control

Functional testing

All components are functionally tested in RNaseAlert Lab Test following the procedure in this protocol. The kit is shown to detect RNase A by analyzing assay results in a fluorometer.

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.

J. Safety Information

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.

Obtaining the MSDS

To obtain Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion:

- At **www.appliedbiosystems.com**, select **Support**, then **MSDS**. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest.
- At **www.ambion.com**, go to the web catalog page for the product of interest. Click **MSDS**, then right-click to print or download.
- E-mail (MSDS_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 MSDSs unless you request fax or postal delivery. Requests for postal delivery require 1–2 weeks for processing.

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

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