Detecting RNase Contamination Using the Quantus™ Fluorometer with the RNaseAlert® Lab Test Kit v2

Alex Sliwicki and Brad Hook
Promega Corporation

Materials Required

- Quantus™ Fluorometer (Cat.# E6150)
- 0.5ml PCR Tubes (Cat.# E4941, E4942)
- RNaseAlert® Lab Test Kit v2 (Ambion Cat.# 4479768)
- RNase A (10mg/ml; Fermentas Cat.# EN0531)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocol: *Quantus™ Fluorometer Operating Manual* #TM396 is available at: **www.promega.com/protocols/**

Introduction

When working with RNA, there is a constant threat of sample degradation due to ribonuclease (RNase) contamination. You must take care during sample preparation as well as downstream applications to avoid contaminating the RNA sample with RNase. Small amounts of RNases present in buffers, on pipette tips and in other reagents used in RNA assays can degrade the sample and compromise downstream assays (1). You can help ensure success in these downstream applications by determining if RNases are present in samples prior to use.

The RNaseAlert® Lab Test Kit v2 from Ambion can detect the presence of RNase in samples. The assay is based on a modified RNA oligonucleotide substrate that emits green fluorescence when cleaved by an RNase. Fluorescence can be measured using the Quantus™ Fluorometer with the blue filter set (excitation: 495nm shortpass up to 495nm; emission: 510–580nm).

Protocols

RNaseAlert® Lab Test Kit v2 Protocol

1. For each sample to be tested, resuspend one tube of RNaseAlert® Substrate v2 with 5µl of 10X RNaseAlert® Lab Test Buffer. Include tubes for negative and positive controls.
2. Add up to 45µl of test solution to each tube, and vortex to mix. Add nuclease-free water to the negative control tube. Add 40µl of nuclease-free water and 5µl of RNase A (provided in the kit) for the positive control tube.

   Note: There are alternative methods to perform the positive control. See the RNaseAlert® Lab Test Kit v2 literature for more information.
3. Transfer 50µl of substrate + sample to a thin-walled PCR tube (Cat.# E4941).
4. Incubate samples for 30–60 minutes at 37°C, limiting exposure to light.
5. Dilute samples to 200µl using nuclease-free water.

6. Measure fluorescence using the Quantus™ Fluorometer with the blue filter by selecting “Tools” on the Home menu, then “Raw Measurement”, and pressing “Blue”. Record RFU values.

Note: Samples with at least two- to threefold more fluorescence than the negative control are considered RNase-contaminated per RNaseAlert® Lab Test Kit v2 literature.

Results

A twofold dilution series of picogram amounts of RNase A from Fermentas was assayed using the RNaseAlert® Lab Test Kit v2. All amounts of RNase A gave a positive result except 0.3pg RNase A (i.e., at least twofold higher than negative control). When the amount of RNase A present in the reaction was plotted against fluorescence, detection was linear at lower RNase A amounts (1.56–12.5pg), but plateaued at higher amounts of RNase A (>12.5pg) due to the limitations of the assay (Figure 1). Because RNase A contamination is detected as a fold change above background, these plateau values are still useful for verifying if a sample contains RNase.

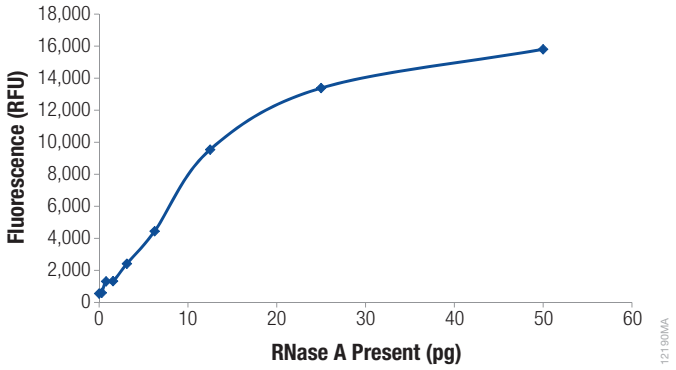

Figure 1. Amount of RNase A detected using RNaseAlert® Lab Test Kit v2 with the Quantus™ Fluorometer. RNase A (Fermentas) was serial diluted twofold in nuclease-free water (0.3–50pg; final amount in reaction) and measured using RNaseAlert® Lab Test Kit v2 with the Quantus™ Fluorometer. Samples are considered contaminated when fluorescence is at least two- to threefold higher than that of the negative control per manufacturer literature.

Conclusion

The RNaseAlert® Test Kit v2 from Ambion is compatible with the Quantus™ Fluorometer and blue filter set for detecting RNase contamination of samples.

Reference

1. Hendricksen, A., Hook, B. and Schagat, T. (2010) RNase contamination happens; Recombinant RNasin® Inhibitor can safeguard your samples. *PubHub* www.promega.com/resources/pubhub/rnase-contamination-happens-recombinant-rnasin-inhibitor-can-safeguard-your-samples/

PROMEGA CORPORATION • 2800 WOODS HOLLOW ROAD • MADISON, WI 53711-5399 USA • TELEPHONE 608-274-4330
www.promega.com • ©2014 PROMEGA CORPORATION • ALL RIGHTS RESERVED • PRICES AND SPECIFICATIONS SUBJECT TO CHANGE WITHOUT PRIOR NOTICE • PRINTED IN USA 4/14 • 12753597 • PART #AN269