



In Vitro Nuclease Detection

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1. Introduction

Deoxyribonucleases (DNases) and Ribonucleases (RNases) are ubiquitous enzymes that catalyze the degradation of nucleic acids primarily through the hydrolysis of phosphodiester bonds (Table 1). While many nucleases have become valuable laboratory reagents, they are also a source of great concern in virtually all molecular biology laboratories, particularly those working with RNA. Over the past few years an even greater premium has been placed on detection and control of nucleases in the laboratory due to advances in techniques for assessing gene expression, such as Real-Time PCR and Microarrays, the development of powerful methods for *in vivo* and *in vitro* gene silencing via antisense constructs and RNA interference, and the discovery of micro-RNAs.

In this report we review two oligonucleotide-based reagents specifically designed to provide unequivocal, high sensitivity detection of nucleases: DNaseAlert™ and RNaseAlert™. We also provide information on reducing the risks of nuclease contamination in the laboratory.

Table 1
Specificity of Various Nucleases

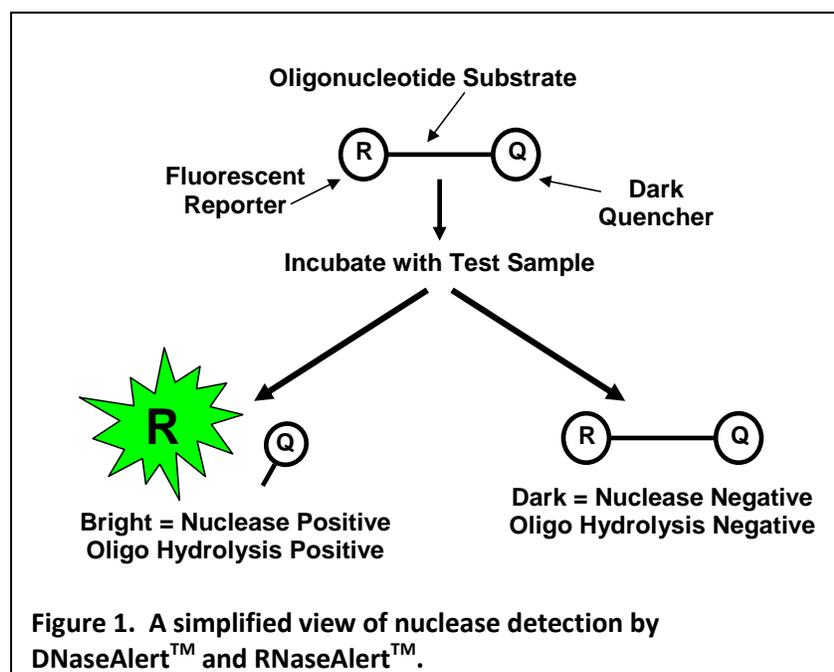
Enzyme	Target	Specificity
<u>Exonucleases</u>		
Snake Venom phosphodiesterase	DNA and RNA	Processive from 3'-end
Spleen phosphodiesterase	DNA and RNA	Processive from 5'-end
<u>Endonucleases</u>		
RNase A	RNA	3'-PO ₄ of pyrimidines (Py)
<i>B. subtilis</i> RNase	RNA	3'-PO ₄ of purines (Pu)
RNase T ₁	RNA	3'-PO ₄ of Guanine
RNase T ₂	RNA	3'-PO ₄ of Adenine
RNase H	RNA	RNA:DNA duplexes
DNase I	DNA	Between Py and Pu
DNase II	DNA	Oligo products
Nuclease S1	DNA and RNA	ss but not ds nucleic acids

Adapted from Garrett and Grisham [1]

2. DNaseAlert and RNaseAlert

RNase and DNase contamination is a major concern for molecular biology laboratories. Contamination can lead to loss of time, money, and data. Integrated DNA Technologies, in collaboration with Ambion, Inc., has developed two nuclease-specific reagents, one for DNase detection and one for RNase detection. Both reagents combine high sensitivity with ease of use in a single tube or plate-based assay.

A combination of a DNase- or RNase-specific oligonucleotide substrate with quenched fluorescence will allow simultaneous nuclease-specificity, assay sensitivity, and ease of use. The principle of both assays is the same (Figure 1). The DNase- or RNase-specific substrate is flanked with a short wavelength fluorescent reporter on the 5'-end and a dark quencher molecule on the 3'-end. In DNaseAlert, the reporter is HEXTM (Hexachlorofluorescein) which has a peak emission at 555nm. In RNaseAlert, the reporter is the parent molecule fluorescein with a peak emission at 520nm. In their intact form both substrates serve as a tether between the reporter molecule and the dark quencher which results in the entire construct remaining dark. However, in the presence of a DNase, in the case of DNaseAlert, or an RNase, in the case of RNaseAlert, the oligonucleotide tether is cleaved and unbound, unquenched reporter is released into solution. Excitation with short wavelength ultraviolet, uV, radiation will cause the free reporter to emit visible light.



Increased fluorescence in both DNaseAlert and RNaseAlert in the presence of an appropriate nuclease is unmistakable. Moreover, both substrates display excellent sensitivity. The DNaseAlert substrate is cleaved in the presence of micrococcal nuclease, mung bean nuclease, S1 nuclease, Bal31 nuclease, Benzonase[®], T7 endonucleases, and exonuclease III. Moreover, the substrate is susceptible to nuclease sources such as those found in body fluids. Experiments with purified DNase I indicate that amounts as low as 5×10^{-3} units (~ 10 pg) are detectable.

The RNaseAlert substrate has likewise been engineered to detect a wide range of nucleases including RNase A, RNase T1, RNase 1, angiogenin, micrococcal nuclease, mung bean nuclease, S1 nuclease, and Benzonase[®]. Experiments with purified RNase A show that amounts as low as 3.5×10^{-7} units (~0.5pg) are detectable. Finally, because DNaseAlert and RNaseAlert have different short wavelength reporters, they can be used together to simultaneously detect the full range of nucleases.

3. An RNase-free Laboratory Environment

As noted, RNases can be a source of worry and wasted time and money. Thus, it is imperative that an RNase-free laboratory be established and maintained for those whose research involves using RNA. Ambion, Inc. has provided a convenient check-list of potential sources of RNase contamination:

Ten Sources of RNase Contamination

Adapted from “Ten Sources of RNase Contamination” (see www.ambion.com).

"Fingerases", such as perspiration, are rich in RNase activity. Thus, the use of ungloved hands could easily result in RNase contamination compromising critical experiments. Use gloves and make it a point to switch to a new pair of gloves frequently during experiments.

Tips & Tubes

Tips and tubes can be an easily overlooked source of RNase contamination. Merely autoclaving will not destroy all RNase activity, since these enzymes are very robust and can regain partial activity upon cooling to room temperature. Always use tips and tubes that have been tested and certified to be RNase-free.

Water and Buffers

Due to the ubiquitous nature of RNases, the water and buffers used in molecular biology applications can be frequent sources of RNase contamination. DEPC-treatment is the most common method used to inactivate RNases in water and buffers. However, certain reagents such as Tris cannot be DEPC-treated. For these types of reagents, it is important to purchase products which are tested and certified to be RNase-free.

Laboratory Surfaces

Contact with laboratory benchtops, glassware, plasticware, and other surfaces that are left exposed to the environment can lead to the introduction of RNase contamination into crucial experiments. These surfaces get contaminated due to the presence of bacterial and fungal spores present in many laboratory environments. Likewise, dead cells shed from human skin can also lead to contamination of exposed surfaces. These surfaces can be treated with an RNase decontamination solution.

Endogenous RNases

All tissue samples contain endogenous RNases. In lieu of immediate processing, liquid nitrogen is often used to rapidly freeze tissues after harvest to minimize RNA degradation. However, freezing tissue in liquid nitrogen is not always convenient, especially if large numbers of samples need to be preserved. Longer-term storage buffers have been developed for this purpose.

RNA Samples

Small amounts of RNases that may co-purify with isolated RNA can compromise downstream applications. Such contamination can also be introduced from tips, tubes, and other reagents used in these procedures. RNase inhibitors are commonly used as a precautionary measure in most enzymatic manipulations of RNA to keep such contaminants in check.

Plasmid Preps

Plasmid DNA used for in vitro transcription and coupled transcription:translation reactions can introduce RNase contamination into reactions. Many researchers degrade the RNA in plasmid preps by RNase treatment. If this procedure has been used, a Proteinase K treatment is recommended followed by a phenol:chloroform extraction to eliminate all traces of RNase prior to subsequent reactions. If the DNA template has been linearized by restriction enzyme digestion, a similar treatment is recommended, since restriction enzymes may be contaminated with RNases.

RNA Storage

The presence of trace amounts of RNase can compromise RNA integrity even if the samples are stored frozen in an aqueous environment. The best method to preserve isolated RNA for long-term storage is to perform a salt/alcohol precipitation and store the nucleic acid as a precipitate in this solution. The low temperature and the presence of alcohol inhibit all enzymatic activity. The acidic pH (due to the presence of sodium acetate or ammonium acetate) also helps stabilize the RNA. Note that the RNA will have to be centrifuged out of this solution prior to any downstream application.

Chemical Nucleases

Though RNase contamination is most commonly suspected whenever RNA degradation is observed, RNA molecules can also undergo strand scission when heated in the presence of divalent cations such as Mg^{2+} or Ca^{2+} at $>80^{\circ}C$ for 5 minutes or more. Thus, a chelating agent should be present whenever there is a requirement for heating RNA.

Enzymes

Both commercially purchased and laboratory prepared enzymes can be a potential source of RNase contamination. RNaseAlert™ can be used to determine the extent of RNase contamination in commercially available enzymes.

4. References

1. Garrett RH and Grisham GM,. (1999) *Biochemistry*. 2 ed. Philadelphia: Brooks Cole.