

## ***In Vitro* Nuclease Detection Quick Look**

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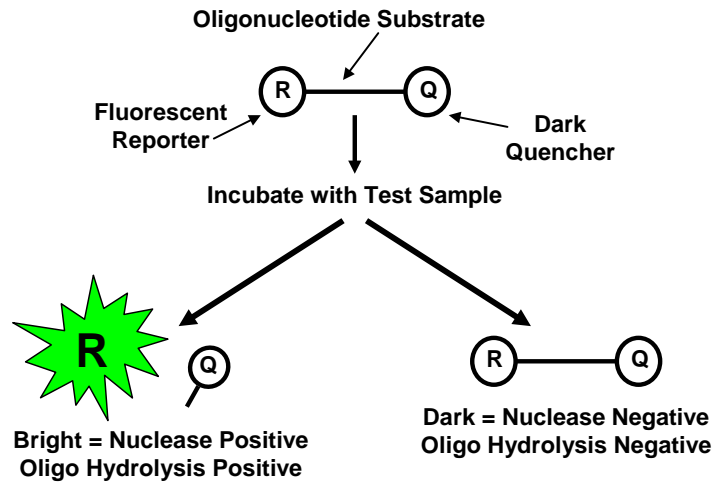
This is a modified, quick look version of the full Technical Report “*In Vitro* Nuclease Detection.” Please see the full version for a more comprehensive explanation and additional ways to create an RNase-free laboratory environment.

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Deoxyribonucleases (DNases) and Ribonucleases (RNases) are ubiquitous enzymes that catalyze the degradation of nucleic acids primarily through the hydrolysis of phosphodiester bonds. 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.

IDT, in collaboration with Ambion, Inc., has developed two nuclease-specific reagents, one for DNase detection and one for RNase detection. Both are specifically designed to provide unequivocal, high sensitivity detection of nucleases with ease of use in a single tube or plate-based assay.

Nuclease-specificity, assay sensitivity, and ease of use are simultaneously achieved through the combination of a DNase- or RNase- specific oligonucleotide substrate with quenched fluorescence. 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. The reporter for DNaseAlert™ is HEX™ (Hexachlorofluorescein), which has a peak emission at 555nm, and the reporter for RNaseAlert™ fluorescein, which has a peak emission at 520nm. In their intact form, both substrates serve as a tether between the reporter molecule and the dark quencher which causes the entire construct to remain dark. However, in the presence of a DNase, for DNaseAlert, or an RNase, for RNaseAlert, the oligonucleotide tether is cleaved and free, 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. Experiments with purified DNase I indicate the amounts as low as  $5 \times 10^{-3}$  units ( $\sim 10\text{pg}$ ) are detectable. Experiments with purified RNase A show that amounts as low as  $3.5 \times 10^{-7}$  units ( $\sim 0.5\text{pg}$ ) 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.

Please see the DNaseAlert or RNaseAlert user guides for more information on these products.