

# *DNaseAlert™ Substrate*

Nuclease Detection System

User's Manual



# DNaseAlert™ Substrate Nuclease Detection System

Nucleases are widely present in the laboratory environment and can interfere with many experiments. IDT has developed DNaseAlert and RNaseAlert Substrate Nuclease Detection Systems for rapid, sensitive detection of DNases and 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 DNaseAlert Substrate is a synthetic DNA oligonucleotide that has a Hex™, reporter dye (hexachlorofluorescein, 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 nucleases; it contains domains that will react with single-stranded endonucleases, single-stranded exonucleases, and double-stranded nucleases. Intact, the substrate has little or no fluorescence. When cleaved by a DNase, the substrate fluoresces pink (536 nm or UV excitation, 556 nm emission) and can be detected visually or using a fluorometer (Figure 1).

Use of DNaseAlert is fast and simple. Lab surfaces or liquid reagents can be tested and verified as being “nuclease-free” or “contaminated” in less than an hour. For speed and ease of use, a simple tube-based visual assay can be performed directly at the site of testing. Alternatively, quantitative fluorescent results (cuvette or micro titer plate formats) can be obtained and used to document nuclease testing for GLP or manufacturing needs.

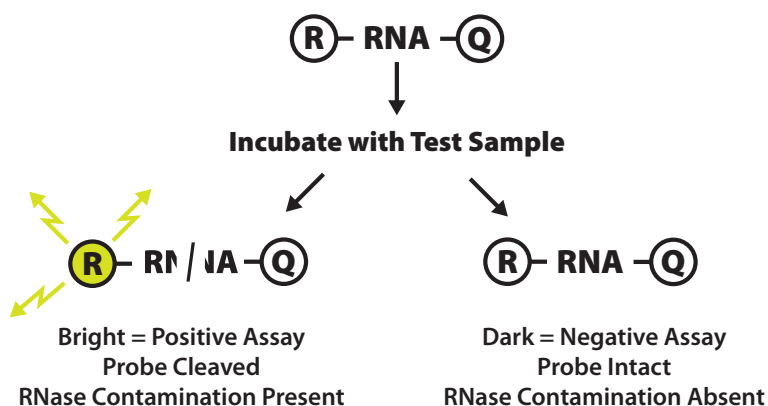


Figure 1. DNaseAlert Assay

## DNaseAlert™ Kit Components

- 25 single-use tubes DNaseAlert Substrate, 50 pmoles per tube
- 250 µL DNaseAlert Buffer
- 2 mL Nuclease-free Water
- 25 µL DNase 1 Enzyme (positive control)
- 50 mL Nuclease Decontamination Solution

DNaseAlert Substrate should be stored long term at -20°C in the dark to prevent photobleaching.

Nuclease Decontamination Solution should be stored at room temperature.

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

## DNaseAlert™ Substrate

25 single-use tubes, 50 pmoles per tube

or

2 tubes bulk substrate, 2 nmoles per tube (100 µL fluorometric assays or 40 visual assays per tube)

## DNaseAlert™ Protocol

### Before Starting

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

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

# DNaseAlert™ Substrate Nuclease Detection System

## Assay Setup

Single-use tubes, testing a liquid solution

1. Resuspend bulk substrate with 200  $\mu\text{L}$  of nuclease-free water (10  $\mu\text{M}$ ).
2. Add 5  $\mu\text{L}$  DNaseAlert Substrate (50 pmoles) into a small, clear-walled microtube. Add 5  $\mu\text{L}$  10X DNaseAlert Buffer to the tube. Set up one DNaseAlert assay for each reagent being tested. Include 2 two control tubes. Thus, if testing 8 samples, set up 10 tubes (8 unknowns plus 1 negative and 1 positive control).
3. Add 40  $\mu\text{L}$  sample to the tube. Mix well. For both control tubes, add 40  $\mu\text{L}$  water (provided with kit); also, add 1  $\mu\text{L}$  nuclease, such as DNase 1, to the positive control tube. The final concentration of substrate is 1  $\mu\text{M}$  for the visual assay; lower substrate concentrations can be used if fluorometric detection is employed (see below).
4. Incubate 30 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).
5. Quick visual confirmation of results: Place tube on a short-wave (300 nm) UV transilluminator.

If the tube remains clear, the assay is negative and sample is free of detectable DNase contamination. If the tube glows pink, DNase contamination is present. A long-wave (365 nm) UV source can be used but sensitivity will be lower.

**Important Safety Note:** never look directly into a UV light source. Always employ protective eye shielding.

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

**Quantitative results:** The DNaseAlert assay is linear over time and can be used for quantitative enzyme activity measurements. Place contents in a nuclease-free cuvette or microtiter plate and read using a fluorometer on "Hex" channel, using 536 nm excitation and 556 nm emission settings. After Step 4 incubation is complete, the sample can be diluted using up to 2 mL Nuclease-free Water as needed, depending on the size of the detection chamber.

**Assessing controls:** If the negative control tube (Nuclease-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 any further assays.

6. Validate all negative assays by adding 1  $\mu\text{L}$  DNase I (or other nuclease) 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 DNase contamination using the DNaseAlert Substrate Nuclease Detection System. 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 nuclease-free filter paper. Pre-wetting the filter paper with Nuclease-free Water will improve sensitivity. After wiping down the surface of interest, soak the filter paper in a small amount of Nuclease-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 substrate by adding 1.0 mL Nuclease-free Water to the tube and mixing well. This will result in a substrate concentration of 2  $\mu\text{M}$ , or 20 pmoles of substrate in 10  $\mu\text{L}$ .  
While bulk substrate can be used in individual tubes, it is most convenient to perform the assay in microtiter plates. IDT recommends use of opaque black plates that minimize scatter and cross-talk between wells.
2. Add 10  $\mu\text{L}$  DNaseAlert 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  $\mu\text{L}$  10X DNaseAlert Buffer to each well. Add 80  $\mu\text{L}$  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 employed if a more sensitive cuvette fluorometer is employed.
4. Read assay in a fluorometer using Hex channel (536 nm excitation, 556 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  $\mu\text{L}$  stock DNase 1 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 DNase activity (see below).

# DNaseAlert™ Substrate Nuclease Detection System

## Troubleshooting Guide

### False Negatives

#### DNase Inhibitors

Solutions with extreme pH, strong ionic strength, or detergents can block action of DNases, preventing detection of contaminants that are present. It is simple to test to determine if your solution contains a component that inhibits the DNaseAlert assay. Set up a standard assay with your test solution and add 1  $\mu$ L DNase I (or other nuclease). If the assay does not convert to “positive” after 1 hour incubation, then your test solution is incompatible with the DNaseAlert system.

#### Low pH Solutions

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

#### Substrate Loss

The DNaseAlert Substrate is provided lyophilized. The dry pellet can become dislodged 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 DNaseAlert Substrate to light can photobleach the fluorescent dye and decrease assay sensitivity. Store bulk substrate and assay tubes in the dark.

#### Nuclease-Substrate Incompatibility

The DNaseAlert Substrate contains a fluorescent dye at the 5'-end and a dark fluorescence quencher at the 3'-end. The action of some exonucleases is blocked by terminal end-groups and, thus, such enzymes will not be detected using this assay (such as E.coli Exo I).

### 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 DNaseAlert Substrate contains DNA bases that will depurinate and degrade when exposed to acid conditions. Avoid contact with solutions < pH 4.5.

#### Substrate Stimulation

Certain organic solvents will disrupt quenching. In particular, DNaseAlert always glows in acetonitrile.



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