DNaseAlert™ Substrate Nuclease Detection System

For Research Use Only. Not for use in diagnostic procedures. | Version 3

REVISION HISTORY

Version	Release date	Description of changes
3	January 2023	Updated to internal MAPSS standards.
2	April 2020	Protocol template update.
1	March 2015	Initial release.

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INTRODUCTION

Nucleases are widely present in the laboratory environment and can interfere with many experiments. IDT developed the DNaseAlert Substrate Nuclease Detection System for rapid detection of DNases. The included DNaseAlert Substrate contains fluorescence-quenched oligonucleotide probes that fluoresce after DNase degradation. The results can be read visually or measured and quantified using a fluorometer. DNaseAlert reagents can be used qualitatively or quantitatively to test lab reagents, work surfaces, equipment, and supplies for nuclease contamination.

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 (Figure 1). 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).

Using the DNaseAlert kit is fast and simple. Lab surfaces or liquid reagents can be tested and demonstrated as "DNase-free" or "contaminated" in less than one hour. For speed and ease of use, a simple tube-based visual assessment can be performed directly at the site of testing. Alternatively, quantitative fluorescent results (cuvette or microtiter plate formats) can be obtained.

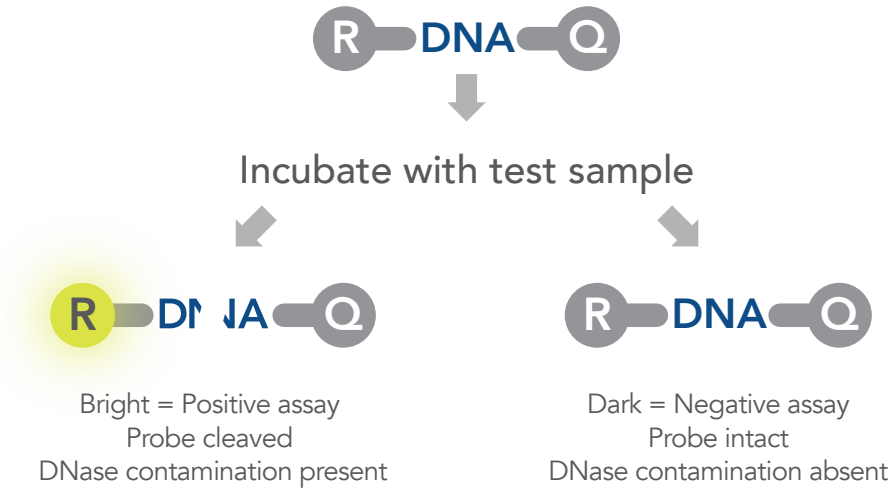

Figure 1. How DNaseAlert Substrate works.

CONSUMABLES AND EQUIPMENT

DNaseAlert Kit contents—Catalog # 11-02-01-04

Component	Quantity	Storage
DNaseAlert Substrate	25 single-use tubes (50 pmol per tube)	–20°C (protect from light to prevent photobleaching)
DNaseAlert Buffer	250 µL	–20°C
Nuclease-Free Water	2 mL	Room temperature
DNase I Enzyme (positive control)	25 µL	–20°C
Nuclease Decontamination Solution	50 mL	Room temperature

Consumables—IDT

Item	Catalog #
DNaseAlert Kit*	11-02-01-04
For liquid solution testing in bulk: • DNaseAlert Substrate, 2 bulk tubes (2 nmol per tube)	11-04-02-04

* DNaseAlert Kit components can be purchased separately. Visit the DNaseAlert product page for all ordering options.

Consumables—Other suppliers

Item	Supplier	Catalog #
Nuclease-free tips and pipettes ranging from 1–1000 µL	Various suppliers	Varies
For liquid solution testing in bulk, choose one: • Nuclease-free black, opaque 96-well plate (recommended) • Other nuclease-free 96-well plate or tubes		
Nuclease-free cuvette (optional)		

Equipment

Item	Supplier	Catalog #
Incubator or water bath capable of 37°C incubation (recommended)	Various suppliers	Varies
UV light source for visual detection: • UV transilluminator [300 nm (recommended) or 365 nm)]		
Fluorometer (for quantitative measurement)		

Avoid cross-contamination

Important! Before you start follow these instructions as best practices:

- Use gloves when handling kit components and performing the DNase detection tests.
- Perform all steps under nuclease-free conditions:
  - Use nuclease-free pipette tips and tubes.
  - If necessary, clean pipettes and other lab surfaces with Nuclease Decontamination Solution before use.

PROTOCOL

Test a liquid solution for DNases with single-use tubes

Note: DNaseAlert Kits contain all reagents needed to perform this method. Prepare one DNaseAlert Substrate single-use tube for each sample to be tested. Include 2 additional tubes for the positive and negative controls.

1. Add 5 µL of Nuclease-Free Water to each DNaseAlert Substrate single-use tube needed for samples and controls.
2. Add 5 µL of 10X DNaseAlert Buffer to each tube.
3. Add 40 µL of sample liquid to each sample tube. Add 40 µL of Nuclease-Free Water to each control tube. Add 1 µL of DNase I to the positive control tube. Mix thoroughly.

   Note: The final concentration of substrate is 1 µM for quick visual assessment; lower substrate concentrations can be used for fluorometric detection (see Quantitative measurement, below).

4. Incubate at 37°C for 30–60 min.

   Tip: 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).

5. Perform detection visually or with quantitative measurement.

   Quick visual assessment: Place tube on a shortwave (300 nm) UV transilluminator. For best results, use a darkened room. If the tube remains clear, the test is negative and the sample is free of detectable DNase contamination. If the tube glows pink, DNase contamination is present.

   Important! Never look directly into a UV light source. Always use protective face shielding.

   Quantitative measurement: Place contents in a nuclease-free cuvette or microtiter plate. Measure fluorescence using a fluorometer set to the HEX channel (536 nm excitation, 556 nm emission). After the incubation at Step 4 (above) is complete, the sample can be diluted with up to 2 mL of Nuclease-Free Water, as needed, to accommodate the size of the detection chamber.

6. Check for potential false negatives. Add 1 µL of DNase I (or other DNase) to each negative sample tube. Mix and incubate at 37°C for 10 min. Repeat Step 5 (perform detection). All negative tubes should now be positive. Any samples that fail to glow during this step must be considered "failed" and the DNase detection test should be repeated. See Troubleshooting for help.

Test a solid object or dry surface for DNases with single-use tubes

Note: DNaseAlert Kits contain all reagents needed to perform this method. Prepare one DNaseAlert Substrate single-use tube for each sample to be tested. Include 2 additional tubes for the positive and negative controls.

1. Add 5 µL of Nuclease-Free Water to each DNaseAlert Substrate single-use tube needed for samples and controls.
2. Add 5 µl of 10X DNaseAlert Buffer to each tube.
3. Add 40 µL of Nuclease-Free Water to each tube. Mix thoroughly.

   Important! If object or surface is too large to dip directly into a sample tube, do not add Nuclease-Free Water to sample tubes yet (add to control tubes only) and proceed to step 4.

4. Place the object or liquid (see Note below) to be tested into a sample tube. Add 1 µL of DNase I to the positive control tube.

   Note: Pipette tips, electrodes, or other small solids can be dipped directly into the prepared reagents. To test surfaces that cannot be dipped into a sample tube:

   - Wipe the surface of interest with a piece of nuclease-free filter paper pre-wetted with Nuclease-Free Water.
   - Soak the filter paper in a small amount of Nuclease-Free Water.
   - Transfer 40 µL of the liquid to a sample tube and mix thoroughly.

5. Incubate at 37°C for 30–60 min.
6. Perform detection visually or with quantitative measurement.

   Quick visual assessment: Place tube on a shortwave (300 nm) UV transilluminator. For best results, use a darkened room. If the tube remains clear, the test is negative and the sample is free of detectable DNase contamination. If the tube glows pink, DNase contamination is present.

   Important! Never look directly into a UV light source. Always use protective face shielding.

   Quantitative measurement: Place contents in a nuclease-free cuvette or microtiter plate. Measure fluorescence using a fluorometer set to the HEX channel (536 nm excitation, 556 nm emission). After the incubation at Step 5 (above) is complete, the sample can be diluted with up to 2 mL of Nuclease-Free Water, as needed, to accommodate the size of the detection chamber.

7. Check for potential false negatives. Add 1 µL of DNase I (or other DNase) to each negative sample tube. Mix and incubate at 37°C for 10 min. Repeat Step 6 (perform detection). All negative tubes should now be positive. Any samples that fail to glow during this step must be considered "failed" and the DNase detection test should be repeated. See Troubleshooting for help.

Test liquid solutions with DNaseAlert Substrate bulk tubes

Note: In addition to the DNaseAlert Kit contents, this bulk testing method requires DNaseAlert Substrate (2 bulk tubes, 2 nmol per tube; catalog # 11-04-02-04). Bulk testing can be performed in individual tubes; however, it is more convenient to use microtiter plates. We recommend using black opaque plates that minimize scatter and cross-talk between wells.

Important! Always include negative and positive control wells. Use duplicate or triplicate sample wells for quantitative measurements.

1. Add 1 mL of Nuclease-Free Water to the bulk DNaseAlert Substrate tube. Mix thoroughly. Final substrate concentration is 2 µM (20 pmol substrate in 10 µL).
2. Add 10 µL of DNaseAlert Substrate to each sample and control well.
3. Add 10 µL of 10X DNaseAlert Buffer to each well.
4. Add 80 µL of sample to the sample wells. Add 80 µL of Nuclease-Free Water to each control well. Add 1 µL of DNase I to the positive control well. Mix thoroughly.

   Note: The recommended final concentration of substrate is 200 nM for a standard 96-well fluorometer; more dilute solutions can be used with some other types of cuvette fluorometers.
5. Incubate at 37°C for 30–60 min.
6. Read results with fluorometer using the HEX channel (536 nm excitation, 556 nm emission). Results can be read as simple endpoint measurements or examined in real time to obtain quantitative kinetic curves.
7. Check for potential false negatives. Add 1 µL of DNase I (or other DNase) to each negative sample tube. Mix and incubate at 37°C for 10 min. Repeat Step 6 (perform detection). All negative tubes should now be positive. Any samples that fail to glow during this step must be considered "failed" and the DNase detection test should be repeated. See Troubleshooting for help.

TROUBLESHOOTING

Issue	Possible cause	Suggested solution
False negatives	**Presence of DNase inhibitors** Solutions with extreme pH, strong ionic strength, or detergents can block action of DNases, preventing detection of contaminants that are present.	Test your solution for inhibitors. • Set up a single-use tube with your test solution and add 1 µL DNase I (or other DNase). • If the tube does not convert to "positive" after a 1-hour incubation, your test solution is incompatible with the DNaseAlert system.
	Low pH solutions Solutions with pH <7.0 will decrease the efficiency of HEX fluorescence.	If possible, adjust the pH of the test solution to obtain pH >7.0 and <9.0.
	Substrate loss 1. The DNaseAlert Substrate is provided dried down. 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. 2. Prolonged exposure of the DNaseAlert Substrate to light can lead to photobleaching of the fluorescent dye.	1. a. Spin down tubes before opening. b. Perform a positive control test. Always perform a positive control test on single-use tubes with negative results. A positive result in another tube, even from the same kit, is not sufficient. 2. Store bulk substrate and single-use tubes in the dark.
	Nuclease–substrate incompatibility The DNaseAlert Substrate contains a fluorescent dye at the 5′ end and a fluorescence quencher at the 3′ end. Activity of some exonucleases is blocked by terminal end-groups; therefore, those nucleases (e.g., *E. coli* Exo I) cannot be detected using this test.	

Issue	Possible cause	Suggested solution
False positives	**Contamination** Nuclease contamination of tubes, pipette tips, and other lab equipment can lead to false positives.	Perform a negative control test. • A negative control must be included. Fluorescence in the negative control tube indicates contamination in the experimental setup. Obtain new supplies and clean work area. • If contamination is suspected, obtain fresh tubes and pipette tips and clean all lab surfaces with Nuclease Decontamination Solution before repeating the test.
	Quencher exhaustion Prolonged exposure of the substrate to UV light can damage the quencher. A tube left on an intense shortwave (254 nm) UV source can turn "positive" even when no nuclease is present.	Read your results immediately to limit exposure to UV light.
	Substrate degradation The DNaseAlert Substrate contains DNA bases that will be depurinated and degraded when exposed to acidic conditions.	Avoid use of solutions with pH <4.5.
	Substrate stimulation Certain organic solvents disrupt quenching. In particular, DNaseAlert Substrate always glows in acetonitrile.	Avoid use of solutions containing acetonitrile.

DNaseAlert Substrate Nuclease Detection System

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