DNase Alert kit

Product Description:

Deoxyribonuclease (DNase) is an enzyme that is ubiquitously present in the environment and many biological materials. The presence of DNase poses a threat to many molecular biology experiments, as DNAse can degrade DNA. Detection methods such as PCR rely on plastics, chemicals, and solutions that are free of DNase. Published methods for detecting DNase are often time-consuming, non-quantitative, and have low sensitivity. In contrast, the DNase Alert kit system is a rapid and convenient detection method that can detect DNase contamination in 10 minutes or less.

The DNase Alert kit system includes a unique substrate that is labeled with both a fluorescent reporter and a quencher. Due to the proximity of the fluor and quencher, the un-cleaved substrate emits very little fluorescence. When DNase is present, the bond between the fluor and quencher is severed, resulting in a bright green signal when excited at the appropriate wavelength. The degree of substrate cleavage is proportional to the level of DNase contamination and can be monitored by the increase in fluorescence on a fluorometer.

This kit can be used to detect DNase I and other DNA nucleases that cleave double-stranded DNA, as well as enzymes that cut single-stranded DNA, such as Exonuclease III, mung bean nuclease, micrococcal nuclease, Bal31 nuclease, S1 nuclease, T7 endonuclease, etc. Most reaction buffers and solutions used for DNA can be tested with this kit, but some systems are incompatible, such as (1) Gel Loading buffer and other dark solutions: Dark solutions may interfere with the excitation of fluorescence or block its emission, making them incompatible with the DNase Alert kit detection system; (2) Solutions that inhibit DNase activity: Since DNase must be active to be detected, solutions that inhibit DNase activity cannot yield reliable results in the DNase Alert kit detection system. Known common DNase inhibiting solutions include high ionic strength solutions (such as 5 M NaCl, 20 X SSC, 3 M sodium acetate, etc.), solutions with a pH less than 4 or greater than 9, dispersants, detergents, chelating agents, or any solution that denatures proteins (such as SDS, guanidine thiocyanate, urea, EDTA, etc.); (3) Solutions that cause chemical instability of the DNase Alert substrate: Solutions that chemically degrade the DNase Alert substrate are also not suitable for testing with the DNase Alert kit detection system, as they may produce false positive signals. The DNase Alert substrate is unstable in solutions such as those with a pH greater than 9 and corrosive solutions (strong acids, strong bases, bleach).

The kit (500 T) contains reagents sufficient for 5×96 high-throughput assays, allowing for sensitive detection of DNases in a simple and easy-to-use fluorescence assay, providing real-time results, and enabling the certification of plastics, enzymes, solutions, and other biological materials as free of DNase before DNase-sensitive applications such as PCR.

Composition and storage conditions

Components	K1901-100 T	K1901-500 T
DNaseAlert Substrate (2 nmol/tube)	1 tube	5 X 1 tube
10X NucleaseAlert Buffer	2.5 mL	11 mL
DNase I (2 U/μL)	10 μL	50 μL
TE Buffer (pH 7.0)	1.5 mL	6 mL
Nuclease-free Water	10 mL	50 mL
Store Nuclease-free Water at -20°C, 4°C or room temperature, DNaseAlert Substrate the components at -20°C away from light, and the others at -20°C for 12 months.		

Experimental manipulation

In this experiment, DNase I was used as the test object

1. Preparation before the experiment

- Cleaning: RNase, RNA and DNA Remover (#K1143) is recommended for cleaning benches, pipettes, and any plasticware used in experiments that may contain DNases. Simply spray or wipe the surface and wipe it clean with a clean paper towel after 5-10 minutes.
- Prepare DNaseAlert Substrate working solution: Dissolve one tube of DNaseAlert Substrate (2 nmol/tube) in 1 mL of TE Buffer (pH 7.0) on ice.
- Dilute 10X NucleaseAlert Buffer with Nuclease-free Water into 0.1X NucleaseAlert Buffer.

2. Experimental Procedure

2.1 Configure the Reaction Mix reaction system (operation on ice, configure as needed):

Regents	1* well	10* well
2 μM DNase Alert Substrate	10 μL	100 μL
10×Nuclease Alert Buffer	1 μL	10 μL
Nuclease-free Water	79 μL	790 μL
Totals	**90 μL**	**900 μL**

2.2 Setting up the DNase I Standard Curve:

[Note] This step is optional and is suitable for quantitative detection of DNase I when only DNase I is present in the sample or for reference DNase activity detection of other DNases.

Preparation of DNase I standards**: On ice, prepare a total enzyme amount gradient of 1 U, 0.5 U, 0.1 U, 0.05 U, 0.01 U, 0.005 U, 0.001 U as follows:

Dilute DNase I (2U/μL) with 0.1X NucleaseAlert Buffer to the appropriate concentration gradients. For initial testing, set concentrations to 0, 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001 U/μL, and add 10 μL to a 96-well plate, resulting in DNase I amounts of 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 U respectively. Alternatively, set up a suitable DNase I concentration for standard curve establishment.

2.3 Detection system settings

Refer to the table below to sequentially add kit components and samples. For initial testing, the sample can be appropriately diluted.

Reagents	Blank Control	Positive Control	Sample
Reaction Mix	100 μL	90 μL	90 μL
DNase I	0	10 μL	0
Sample	0	0	10 μL
Totals	**100 μL**	**100 μL**	**100 μL**

On ice, Reaction Mix was added to a 96-well plate with a black and white background, followed by different amounts of DNase I, mixed and immediately placed in a microplate reader for detection. For more reliable results, it is recommended to have parallel wells or 3 double wells per sample.

2.4 Microplate reader settings: temperature 37ºC, excitation wavelength 492 nm, emission wavelength 518 nm, reading values every 30 s-1 min, detection for 20 min.

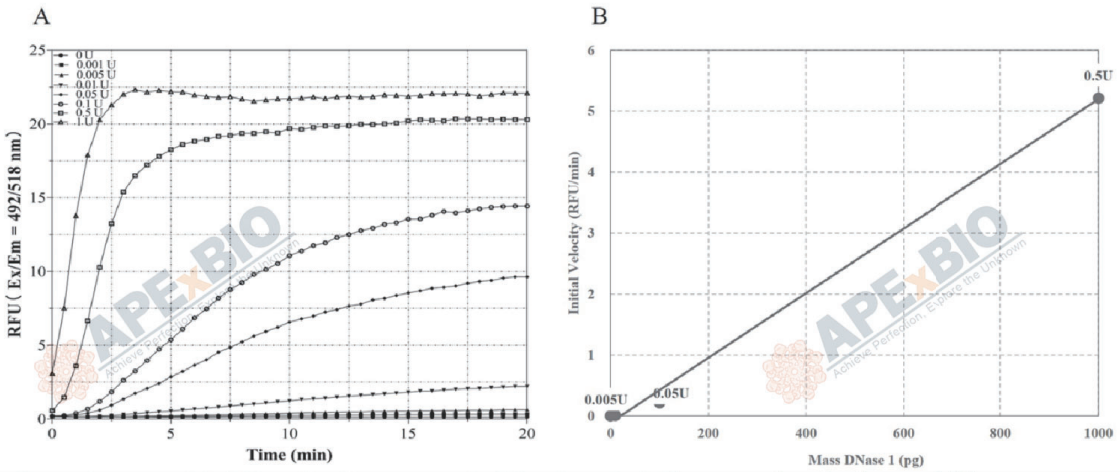
[Note 1]: The continuous detection time can be adjusted according to the DNase activity in the samples to be tested but ensure that data for more than 6 points is obtained. For samples with high DNase activity, it is recommended to set the total detection time to 20 minutes, with an interval of 30 seconds to 1 minute; for samples with very low DNase activity, the total detection time can be extended to 1 hour, with intervals of 5 or 10 minutes.

[Note 2]: If the fluorescence microplate reader does not have temperature control, detection can also be performed at room temperature. However, this will measure the enzyme activity under room temperature conditions, which may be lower than at 37ºC. The degree of reduction in enzyme activity may vary under different experimental conditions.

2.5 Calculation:

Calculate the relative enzyme activity of DNase in the samples based on the detected fluorescence intensity. When performing DNase I detection, the enzyme activity of DNase I in the samples can be calculated using the standard curve plotted and the fluorescence intensity values of the samples. For reference, see the experimental result graph below for DNase I standard detection using this kit. The initial rate calculation is derived only from the data points in the first 1-3 minutes after adding DNase I.

3. Experimental results (DNase I detection result graph)



The performance of the DNase Alert kit system on the DNase I standard. Fluorescence intensity changes of different amounts of DNase I detected by this kit within 20 minutes (A). The initial reaction velocity of different amounts of DNase I was linearly (R>0.99) (B).The actual test data will vary depending on the experimental conditions and testing instruments, and the data in the figure is for reference only.

Precautions

1. This product is for scientific research purposes only.

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