Cat.:BP-16-100

DNase Residual Quantitative Detection Kit (Fluorescent Probe Method)

V01

BIORI BIOTECH

Product Description

Deoxyribonuclease (DNase) is an enzyme capable of hydrolyzing the phosphodiester bonds in DNA molecules. Similar to ribonuclease (RNase), DNase is widely present in laboratory environments and biological materials. Since nucleases can degrade nucleic acids, their presence can interfere with many experiments, making it necessary to assay for DNase.Existing methods for detecting DNase include nucleic acid hydrolysis gel electrophoresis, ultraviolet spectrophotometry, high-performance liquid chromatography (HPLC), and electrochemical methods, among others. However, these methods suffer from issues such as being time-consuming, lacking accurate quantification, having low sensitivity, or being limited by instrumentation. The DNase Residue Quantitative Detection Kit independently developed and launched by Baorui Bio utilizes the principle of fluorescent probe technology, enabling rapid quantitative detection of DNase with advantages such as high sensitivity and simple operation. The DNase substrate used in this kit is a synthetic DNA oligonucleotide probe. One end of the probe is labeled with a VIC fluorophore (also called the donor), and the other end is labeled with a BHQ1 quencher (also called the acceptor). The absorption spectra of these two groups have a certain degree of overlap. When the distance between these two fluorescent groups is appropriate, fluorescence energy is transferred from the donor to the acceptor, resulting in the attenuation of the donor fluorophore's own fluorescence intensity. When this substrate is cleaved by DNase, the two ends of the DNA substrate separate, causing the two groups to move apart. The VIC fluorescence is no longer quenched by BHQ1, allowing the detection of VIC fluorescence. The rate of increase in the fluorescence signal is positively correlated with the amount and activity of the enzyme.

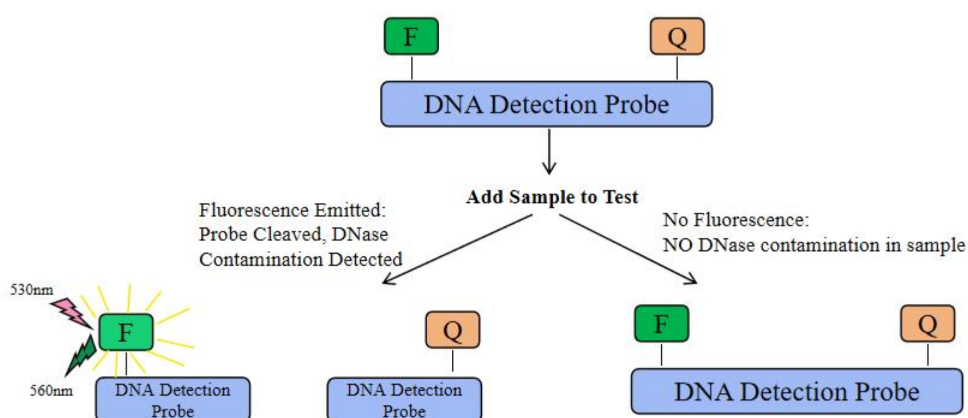

Fig. 1 Principle of DNase residue detection with fluorescent probe-based method

Components

Product Name	Cat. No.	Components	Volume
DNase Residual Quantitative Detection Kit (Fluorescent Probe Method)	BP-E04	DNase I (2 U/μL)	10 μL
	BP-AS-36	DNase Substrate	0.8 mL
	BP-AS-13	10×DNase I Reaction Buffer	1 mL
	BP-AS-11	DNase-free ddH_2O	10 mL

Storage

Store at -20±5°C. Protect DNase Substrate (BP-AS-36) from light.

Materials

It is recommended to use 96-well black plates (DNase-free) and a microplate reader capable of measuring fluorescence intensity(not supplied in this kit).

Protocol

1.Preparation of Reagents

1.1 Before use, equilibrate the 10× DNase I Reaction buffer, DNase substrate and nuclease-free water to room temperature for later use. Keep 2 U/μL DNase I on ice during use and store at -20℃ after use.

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1.2 Dilute the 10× DNase I Reaction buffer to 1× with nuclease-free water. For example, take 500 μL of 10× DNase I Reaction buffer, add 4500 μL of nuclease-free water, and mix thoroughly to obtain 5 mL of 1× DNase I Reaction buffer.

2. Preparation of Testing Samples

Dilute the testing samples to appropriate concentrations using 1× DNase I Reaction buffer. If the concentration range is uncertain during initial detection, perform serial dilutions. Keep on ice for later use.

3. Setting Up DNase I Standard Curve

Dilute 2 U/μL DNase I to appropriate concentration gradients using 1× DNase I Reaction buffer. For initial detection, the concentrations can be set as 0,0.00016,0.00008,0.00004,0.00002,0.00001,0.000005,0.0000025 U/μL. Add 10 μL of each concentration into a 96-well microplate. At this point, the amounts of DNase I are 0,0.0016,0.0008,0.0004,0.0002,0.0001,0.00005,0.000025 U, respectively. Appropriate DNase I concentrations can be customized for standard curve establishment.

4. Setting Up Reactions

Set up the reaction according to the following configuration. It is recommended to set up duplicates or triplicates for each sample/control.

Components	Blank Control	Sample	Positive Control
1×DNase I Reaction Buffer	92 μL	82 μL	82 μL
DNase I	0	0	10 μL
Sample	0	10 μL	0
DNase Substrate	8 μL	8 μL	8 μL
Total Volume	100 μL	100 μL	100 μL

Note: If a DNase I standard curve has been established, it is not necessary to set up a positive control.

5. Detection

5.1 Mix thoroughly by vortexing for 1-2 minutes to ensure complete mixing.

5.2 Immediately perform fluorescence measurement using a fluorescence microplate reader after mixing. Set the microplate reader temperature to 37°C, with excitation wavelength at 530 nm and emission wavelength at 560 nm. Read values every 5 minutes. The continuous measurement duration can be adjusted according to the RNase activity in the test samples, but ensure that at least 6 data points are obtained.

6. Calculation

For DNase I detection, calculate the DNase I enzyme activity in samples using the plotted standard curve and the sample fluorescence intensity values. Refer to Figure 2 for the detection results of DNase I standards obtained with this kit.

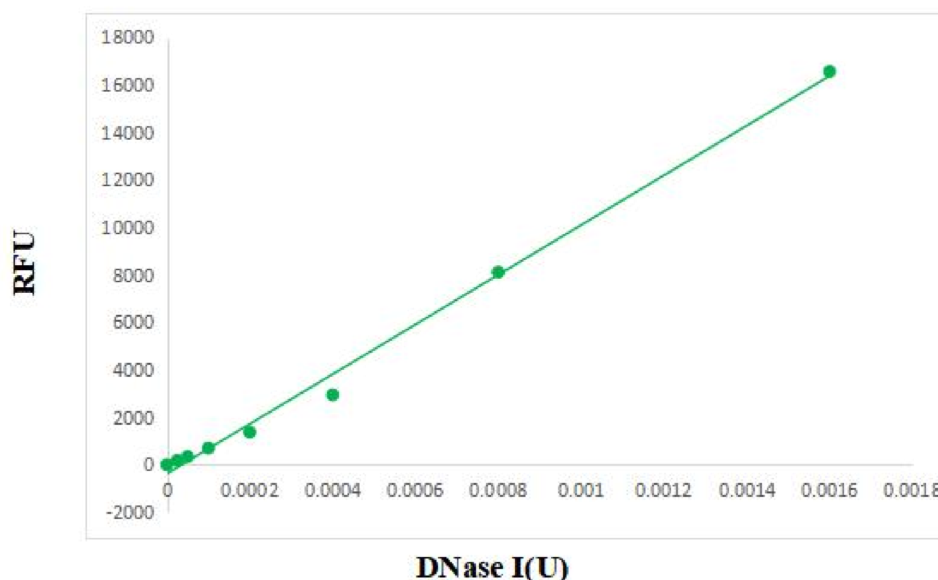

Fig. 2 DNase I standard curve generated with this kit

Notes

1. It is recommended to perform DNase residue detection in a relatively clean environment such as a laminar flow clean bench or biosafety cabinet to prevent the testing samples from being affected by DNase present in the environment.
2. The 10× DNase I Reaction buffer, DNase substrate, and nuclease-free water must be fully equilibrated to room temperature before use. For the first use of each component, it is recommended to centrifuge the liquid to the bottom of the tube before opening the cap.
3. Take precautions during operation to prevent reagent contamination by DNase. If necessary, nuclease decontamination agents may be used to eliminate DNase present in the environment before each experiment.
4. The 96-well black microplates and other consumables required for the experiment must be ensured to be free from DNase contamination.
5. Pay attention to personal protection during the experiment. Please wear a lab coat and disposable gloves when performing operations.
6. Please mix thoroughly before use. Avoid repeated freezing and thawing.