RNase Alert-Plus

Introduction

RNases are a class of hydrolases that catalyze the degradation of ribonucleic acid (RNA) molecules into smaller components in vivo and in vitro. RNase enzymes can be divided into two classes: exoribonucleases and endoribonucleases. The most common RNases include RNase A, RNase T1, RNase H, etc. Among them, RNase A is mainly used to remove contaminated RNA from RNA samples, or improve the sample purity in some RNA purification experiments; RNase T1 can specifically cut guanine residues in RNA, and is widely used in RNA sequence analysis, structural study and preparation of short RNA fragments. RNase H can specifically cut RNA in RNA-DNA hybrids, and has important applications in reverse transcription process, cDNA synthesis, antiviral research and RNA-DNA interaction research.

However, in molecular biology experiments, the presence of RNase may lead to the degradation of RNA samples, thus affecting the experimental results. For example, in the process of RNA extraction, RT-PCR or RNA purification, if the sample is contaminated by RNase, the RNA will become unstable, affecting the accuracy of the data. RNase is ubiquitous in the environment, with high concentrations in some biological materials, and often contaminates common molecular biological reagents such as reaction buffers, reverse transcriptase, RNA polymerase, and buffers used for RNA purification and storage. Since even trace levels of RNase contamination can severely disrupt RNA-related experiments, testing solutions in contact with RNA for RNase is an important precautionary measure before performing RNA manipulation.

RNase Alert-Plus is based on the principle of Fluorescence Resonance Energy Transfer (FRET) and can detect RNase activity with high sensitivity. In the kit, the fluorophore and quencher are closely paired to form an energy transfer pair. When RNase is present, the enzyme cleaves the RNA chain, causing a change in the distance between the fluorophore and the quencher, leading to the release of fluorescence from the fluorophore, which can be detected. By monitoring the changes in the fluorescence signal, RNase activity can be quantitatively analyzed in real-time, providing an efficient and accurate detection method. This kit is widely used in RNA research and enzyme activity analysis.

RNase Alert-Plus is a reagent kit that rapidly, sensitively, and specifically detects RNases such as RNase A and RNase T1 using fluorescence methods. The optimized RNase A substrate probe can detect RNase A activity as low as 0.5 pg, showing a good linear correlation in the range of 0-10 pg, and allows for quantitative measurement of activity through a standard curve. Additionally, this kit can also be used to detect RNase T1 activity, with a range from 10^{-3} U to 1 U.

Components and Storage

Components \ Size	K1903 - 100 tests	K1903 - 500 tests	Storage
RNase Alert Substrate-Plus	1 tube	5 tubes	-20℃, protect from light
10×RNase Alert Buffer	1 mL	5 mL	-20℃
RNase A (10mg/mL)	10 μL	50 μL	-20℃
TE Buffer (pH 7.0)	1.5 mL	6 mL	-20℃
Nuclease-free Water	10 mL	50 mL	Room Temperature
Shipping: Dry Ice	**Shelf life:** 12 months		

Protocol

This experiment uses RNase A as the test subject.

1. Pre-experiment Preparation

- **Cleaning:** Products RNase, RNA and DNA Remover **(Cat. No.K1143)** are recommended for cleaning workstations, pipetters, and any plastic utensils used in the experiment that may contain RNases. Simply spray or wipe the surface and wipe it clean with a clean paper towel after 5-10 minutes.
- Thaw RNase A on ice to maintain enzyme activity. After thawing, gently shake the tube to ensure RNase A is evenly dissolved. After balancing the remaining kit components to room temperature, vortex them to mix well for later use. After use, it is best to immediately store the components according to the kit instructions, especially RNase A.
- **Preparing RNase Alert Substrate-Plus Working Solution:** Perform this step on ice. Dissolve one vial of RNase Alert Substrate-Plus in 1 mL of TE Buffer (pH 7.0) thoroughly.
- Dilute the 10×RNase Alert Buffer with Nuclease-free Water to prepare a 0.1× RNase Alert Buffer. For example, take 10 μL of 10×RNase Alert Buffer and add 990 μL of Nuclease-free Water, then mix thoroughly to obtain 1 mL of 0.1×RNase Alert Buffer.

2. Setting up the RNase A Standard Curve:

[Note: This step is optional and is suitable for the quantitative detection of RNase A when the sample contains only RNase A, or for detecting other RNases by referencing the RNase A enzymatic activity.]

2.1 Prepare the Reaction Mix as shown in the table below:

Components	Volume
RNase Alert Substrate-Plus	110 μL
10×RNase Alert Buffer	10 μL
Nuclease-free Water	870 μL
Total volume	990 μL

2.2 Set up a black 96-well reaction plate with the following steps:

Sample type	Component	Volume
Blank Control	Reaction Mix	100 μL
Positive Control	Reaction Mix	90 μL
Sample	Reaction Mix	90 μL

2.3 Detection: Set the temperature to 37 ℃, with an excitation wavelength of 490 nm and an emission wavelength of 520 nm. Collect 5-10 data points within 10 minutes. (The purpose of reading fluorescence before adding RNase A is twofold: first, to establish the background fluorescence level, and second, to confirm that the buffer and the 96-well plate are free from RNase contamination.)

2.4 Plotting: The standard curve should maintain a near-linear shape to ensure the accuracy of subsequent experimental results.

2.5 Dilute RNase A with 0.1×RNase Alert Buffer to prepare an appropriate concentration gradient. For initial testing, concentrations can be set at 0, 0.05, 0.125, 0.25, 0.5 and 1 pg/μL. Add 10 μL of each dilution to the 96-well plate, corresponding to RNase A amounts of 0, 0.5, 1.25, 2.5, 5 and 10 pg, respectively. Alternatively, adjust the RNase A concentrations to establish the standard curve as needed.

3. Detection system setting

Refer to the following table to add the kit components and samples. For initial testing, the sample to be tested may be diluted appropriately.

Components	Blank Control	Positive Control	Sample
Reaction Mix	100 μL	90 μL	90 μL
RNase A	0	10 μL	0
Sample	0	0	10 μL
Total volume	100 μL	100 μL	100 μL

Operate on ice. After the background value stabilizes to a linear trend, add different amounts of RNase A, mix thoroughly, and immediately place the plate into the microplate reader for detection.

Note: To obtain more reliable results, it is recommended to set up parallel wells or 3 replicates for each sample.

4. Testing

4.1 Mix the above system thoroughly, centrifuge briefly, and ensure there are no air bubbles.

4.2 Microplate Reader Settings: Set the temperature to 37℃, with an excitation wavelength of 490 nm and an emission wavelength of 520 nm. Read the values every 1 minute and perform kinetic detection for 60 minutes.

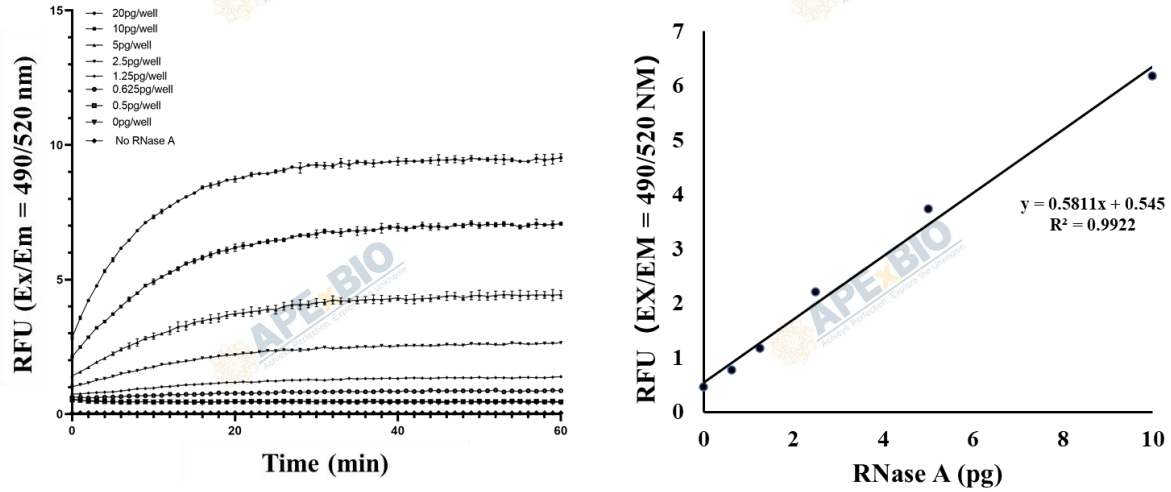
Notes a. The continuous determination time can be adjusted appropriately according to the RNase activity in the sample to be tested, but it is necessary to ensure that more than 6 points of data are obtained.

b. Fruit fluorescent enzyme spectrometer has no temperature control function, it can also be measured at room temperature, but this detection is the enzyme activity at room temperature, at this time the enzyme activity may be lower, different experimental conditions will be lower degree.

5. Calculation

Determine the relative RNase A activity in the sample based on the measured fluorescence intensity. For RNase A detection, the enzyme activity in the sample can be calculated using the standard curve and the sample's fluorescence intensity value. This kit is designed for the detection of RNase A standards, with reference to the experimental result graphs below.

6. Experimental results (RNase A test results diagram)



The performance of RNase Alert-Plus on the RNase A standard. Fluorescence intensity changes of different amounts of RNase A detected by this kit within 60 minutes (left). The effect of different amounts of RNase A standards was detected with an assay time of 20 min ($R^2 > 0.99$) (right). The actual test data will vary depending on the experimental conditions and testing instruments, and the data in the figure is for reference only.

Notes

1. During experiments, all reagents, equipment, and solutions should be RNase-free. Even trace amounts of RNase contamination can interfere with results, leading to unnecessary background fluorescence or degradation.
2. After use, RNase A should be promptly returned to the -20 ℃ freezer, mainly to prevent temperature fluctuations from negatively affecting the enzyme activity.
3. RNase A should not be repeatedly frozen and thawed, as this may lead to enzyme degradation and reduced activity. It is best to take only the required amount and store the remaining portion. If repeated use is necessary, it is recommended to aliquot RNase A into smaller portions to avoid thawing and refreezing the entire vial.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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