Product Description

Ribonuclease (RNase) is a class of nucleases that catalyzes RNA degradation into small fragments. The RNase family includes RNase A, B, C, H, S-RNase, P, T, and others. RNase A is a widely used endonuclease that specifically cleaves phosphodiester bonds on single-stranded RNA at the 3' end of cytidine (C) and uridine (U) residues, producing oligonucleotides with 2',3'-cyclic phosphate termini. Current RNase detection methods include radioisotope assays, spectrophotometry, fluorescence quenching, and electrochemical methods. Biori's RNase Residual Quantitative Detection Kit employs a fluorescent probe-based approach for rapid, high-sensitivity detection of RNase A activity, with lower limit of detection of 0.078 pg RNase. The kit utilizes a synthetic RNA oligonucleotide probe with a FAM fluorophore (Donor) at one end and a TAMRA quencher (Acceptor) at the other. When the two groups are in close proximity, fluorescence energy transfers from donor to acceptor, quenching the donor's fluorescence. Upon cleavage by RNase, the probe separates, the two groups disperse, and FAM fluorescence is no longer quenched, resulting in detectable fluorescence. The rate of fluorescence signal increase correlates positively with enzyme quantity and activity.

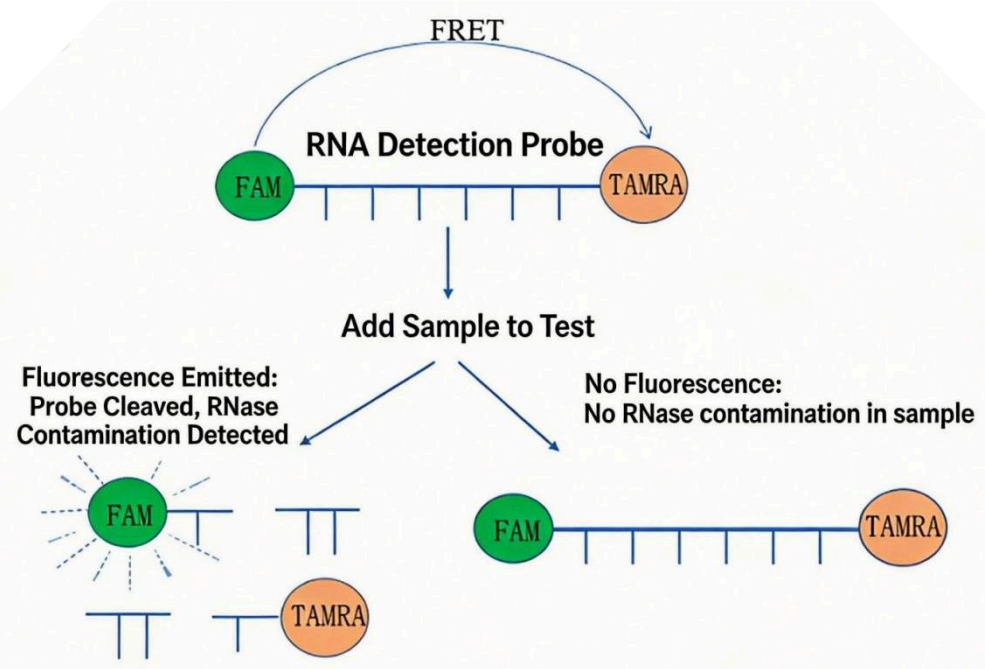

Fig. 1 Principle of RNase residue detection with fluorescent probe-based method

Components

Product Name	Cat. No.	Components	Volume
RNase Residual Quantitative Detection Kit	BP-AS-18	RNase A(10 mg/mL)	10 μL
	BP-AS-19	RNase Substrate	1 mL
	BP-AS-20	10× Reaction Buffer	1 mL
	BP-AS-11	RNase-free ddH_2O	1 mL×5

Storage

Store at -20±5°C . Protect RNase Substrate (BP-AS-19) from light.

Protocol

1.Preparation of Reagents

1.1 Before use, equilibrate the 10× Reaction buffer, RNase substrate and nuclease-free water to room temperature for later use. Keep 10 mg/mL RNase A on ice during use and store at -20℃ after use.

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

2. Preparation of Testing Samples

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

3. Setting Up RNase A Standard Curve

Dilute 10 mg/mL RNase A to appropriate concentration gradients using 1× reaction buffer. For initial detection, the concentrations can be set as 0, 0.0078, 0.0156, 0.0313, 0.0625, 0.125, 0.25, 0.5 and 1 pg/μL. Add 10μL of each concentration into a 96-well microplate. At this point, the amounts of RNase A are 0, 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5 and 10 pg, respectively. Appropriate RNase A 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.

Component	Blank Control	Sample	Positive Control
1× Reaction Buffer	90 μL	80 μL	80 μL
RNase A	0	0	10 μL
Sample	0	10 μL	0
RNase Substrate	10 μL	10 μL	10 μL
Total Volume	100 μL	100 μL	100 μL

Note: If a RNase A 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 490 nm and emission wavelength at 520 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 RNase A detection, calculate the RNase A enzyme activity in samples using the plotted standard curve and the sample fluorescence intensity values. Refer to Fig 2 for the detection results of RNase A standards obtained with this kit.

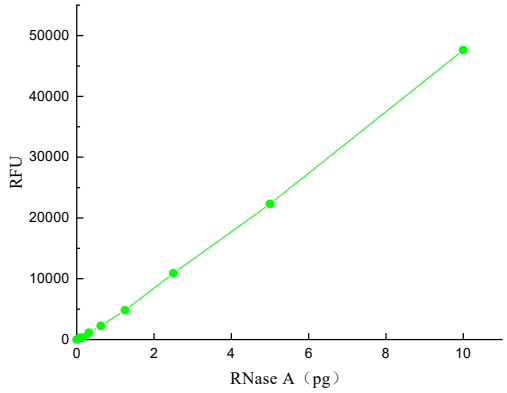

Fig. 2 RNase A standard curve generated with this kit

Notes

1. It is recommended to perform RNase 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 RNase present in the environment.

2. The 10× reaction buffer, RNase 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 RNase. If necessary, nuclease decontamination agents may be used to eliminate RNase 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 RNase contamination.

5. Pay attention to personal protection during the experiment. Please wear a lab coat and disposable gloves when performing operations.

6. Mix thoroughly before use and avoid repeated freeze-thaw cycles.