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RNase Detection Kit

Product Number: DT007

Shipping and Storage

Store at -20±5℃ to avoid repeated freeze-thaw cycles.

Components

Component	DT007
RNase Substrate (20 Reactions/Tube)	2 Tubes
10×RNase Buffer	400μl
RNase A (20μg/μl)	20μl
RNase A Storage Buffer	1ml
RNase Free Water	1ml×5

Description

Ribonuclease (RNase) is widely present in nature, and even trace amounts of RNase can contaminate common molecular biology reagents, especially affecting experiments involving RNA. Therefore, there is a need for an RNase residue detection reagent that can test for RNase residues in any RNA related solution.

The RNase Detection Kit provides a convenient and sensitive fluorescence detection method that can be used to detect the presence or absence of RNase in samples such as proteins, nucleic acids, and reagent solutions. This reagent kit can simultaneously detect RNase A, RNase T1, RNase I, Micrococcus nuclease, S1 nuclease, Mung bean nuclease, and BenzoNuclease ® Multiple RNases, including omnipotent nucleases and RNase R.

RNase Substrate is a novel RNase A substrate, which is labeled with a fluorescent group at one end and a quenched group at the other end. The physical distance of the quenched group suppresses the fluorescence emitted by the fluorescent group to an extremely low level. Through short wave ultraviolet irradiation or detection in a fluorometer, solutions contaminated with RNase will produce green fluorescence during detection, while solutions without RNase will not emit fluorescence.

Features

1. High sensitivity, RNase A detection sensitivity can reach 0.313pg/ml;
2. Convenient and intuitive RNase residue detection;
3. 30 minute rapid detection;
4. Can detect multiple trace amounts of RNase.

Application

Used to detect the presence of RNase residues in the system.

Note

1. When diluting and using RNase A and other related RNases, try to separate them from the experimental buffer and substrate as much as possible to avoid contamination;
2. Each component in the reagent kit should be centrifuged briefly before use;
3. Be careful during use to avoid introducing substances other than the sample;
4. In most cases, reactions with residual RNase will produce significant fluorescence within 10 minutes or less. To achieve optimal results, incubation should be carried out for at least 30 minutes.
5. RNase Substrate in dry powder state can be stored at -20℃ for a long time. When configured as a 5×RNase Substrate Mix, it should be used as soon as possible to avoid repeated freeze-thaw and prolonged exposure to light when opening the lid.

6. The use of RNase Substrate inhibitors or inactivators (such as EDTA, SDS, acid, alkali, high salt, etc.) should be avoided as much as possible in the test sample solution, as these preparations can easily lead to false negative or false positive results.

Protocol

1. Preparation before the experiment

1.1. Before the experiment, the workbench should be cleaned and the required RNase free materials (solution, EP tube, suction head, etc.) should be prepared.

1.2. Take out 10×RNase buffer, completely melt at room temperature, centrifuge instantly, and set aside.

1.3. RNase Substrate is initially in a dry powder state. Before use, centrifuge at 12000 rpm for 30 seconds. Centrifuge the dry powder to the bottom of the tube, add 100μl of RNase Free Water, shake well, and centrifuge instantaneously (both mixing and centrifugation times do not exceed 5 seconds). Then add 100μl of 10×RNase Buffer to prepare a 5×RNase Substrate Mix containing RNase Substrate and 10×RNase Buffer. Mix well and centrifuge instantaneously, and set aside (hereinafter referred to as 5×RNase Substrate Mix).

1.4. According to the experimental requirements, the total concentration of positive control (RNase A) added should be ≥ 5pg. To avoid contamination of the experimental area by RNase A due to operational errors, please dilute RNase A (20μg/μl) to a usable volume in the RNase dedicated experimental area according to the following steps, and wait for use.

Number	Preparation process	Concentration
1	1μl RNase AStock solution + 99μl RNase A Storage Buffer	0.2μg/μl
2	1μl Number 1 Sample + 99μl RNase A Storage Buffer	2×10-3μg/μl
3	1μl Number 2 Sample + 99μl RNase A Storage Buffer	2×10-5μg/μl
4	10μl Number 3 Sample + 90μl RNase A Storage Buffer	2×10-6μg/μl
5	12.5μl Number 4 Sample + 187.5μl RNase Free Water	1.25×10^{-7}μg/μl(125pg/ml)

Note: Except for RNase Substrate dry powder, which is centrifuged at 12000 rpm for 30 seconds, all other reagents and operations are centrifuged at low speed using a handheld centrifuge.

2. Reaction steps

Usually, three sets of reactions are required: blank control, test sample, and positive control. The following experiments are performed in an ice bath:

2.1. Blank control group

Add 10μl of 5×RNase Substrate Mix to the negative group, and then add 40μl of RNase Free Water to supplement the 50μl reaction system.

2.2. Sample group to be tested

Add 10μl of 5×RNase Substrate Mix to the test group, add a certain volume of test solution (≤ 40μl), and finally use RNase Free Water to supplement the system to 50μl.

2.3. Positive control group

Add 10μl of 5×RNase Substrate Mix to the positive group and 40μl of diluted RNase A (RNase T1 and other RNases with different activities can also be added to replace RNase A);

2.4. Gently mix the above reaction system and centrifuge instantaneously (centrifuge time does not exceed 5 seconds).

3. Detection

Incubate at 37℃ for 30-60 minutes

3.1. Detect for the generation of green fluorescence under UV lamp or fluorescence conditions;

3.2. Use real-time quantitative PCR equipment, UV spectrophotometer, enzyme-linked immunosorbent assay (ELISA), and other fluorescence signal detection instruments that meet the excitation/emission wavelength(λex/em)of 490/520nm to detect the fluorescence intensity emitted. The main detection settings of the ELISA instrument are as follows:

Parameter	Set up
Mode	Dynamic testing
test method	Fluorescence

Maximum excitation/emission wavelength	490/520nm
Gain value	Optimal
Fluorescence collection	Intermittent collection, collected every 1-1.5 minutes
Temperature	37℃

4. Data analysis

4.1. UV lamp observation

After the reaction is complete, place the EP tube under a UV lamp, turn on the light source, and observe whether green fluorescence is produced.

Blank control: The solution is transparent and shows no visible fluorescence;

Positive control: The solution shows obvious green fluorescence;

Sample to be tested: If the solution produces green fluorescence, it indicates that the sample is contaminated with RNase. If the solution does not produce fluorescence, it indicates that the residual RNase detection of the sample is qualified.

4.2. Analysis of testing instruments

Record the fluorescence intensity values of each solution after reaction separately:

Blank control: having a lower fluorescence intensity value (different instruments may detect differences in fluorescence intensity values, based on the corresponding testing instrument), used as the fluorescence background or fluorescence background signal of the solution;

Positive control: The fluorescence value is at least 10 times higher than that of the blank control;

Sample to be tested: If the fluorescence value of the solution is twice or more than that of the blank control, it indicates that the sample to be tested has been contaminated with RNase.

FAQ

1. Fluorescence (value) in blank control

The pollution caused by the introduction of RNase in the environment can be solved by the following methods: using materials without RNase as much as possible; Prepare a blank control first, and then prepare a positive control with RNase to avoid introducing RNase contamination into the blank control.

2. The situation where there is no fluorescence (value) produced in the positive control

Possible loss of RNase activity, retest with another batch of RNase with the same or high activity.

3. False negative results in the sample

It should be considered to exclude components in the test sample that inhibit or affect RNase activity, such as RNase Inhibitors, which inhibit the cleavage of probe fluorescent groups.

4. False positives in the sample

Check if there are any components in the test sample that affect the RNase Substrate, causing instability of the probe and breaking of the fluorescent group.

Related products

Product number	Product name
GMP-T701	T7 RNA Polymerase, GMP Grade
E131	T7 High Yield RNA Transcription kit
GMP-M062	Vaccinia Capping Enzyme, GMP Grade
GMP-RI01	RNase Inhibitor, GMP Grade
GMP-M072	mRNA Cap 2'O Methyltransferase, GMP Grade
GMP-DI05	DNase I Recombinant GMP grade
GMP-M012	Poly(A) Polymerase, GMP Grade
GMP-M036	Pyrophosphatase, Inorganic (yeast), GMP Grade