TransDetect® Fluorescence RNase Detection Kit

Please read the datasheet carefully prior to use.

Cat. No. FM611

Storage: at -20°C for two years.

Description

TransDetect® Fluorescence RNase Detection Kit uses fluorescence-based method to rapidly and highly sensitively detect residual RNase in samples. Based on the principle of FRET: RNA probe tagged with a fluorescent reporter at the 5' end and a quencher at the 3' end is used as substrate. In the absence of RNase in sample, the quencher and reporter are near one another, and no fluorescent signal is generated; When RNase is present, the quencher and reporter are spatially separated in solution as RNA substrate is cleaved. This causes a fluorescence signal that can be detected. The RNA probe substrate has been optimized, which can detect as low as 0.3 pg of RNase A and has good detection effect on various RNases, including RNase A, RNase T1, RNase I, micrococcal nuclease, S1 nuclease, mung bean nuclease and Benzonase.

Applications

Suitable for detection of RNase in biological materials, experimental environments, reaction buffers, storage buffers, etc.

Not applicable range

- This kit requires the collection of fluorescence values, so it is not suitable for dark-colored solutions, which may interfere with fluorescence excitation and affect the result determination.
- Solutions that inhibit RNase activity or degrade RNA substrates are not applicable, such as solutions with a pH<4, a pH >9, SDS, EDTA or high salt ions.

Kit Contents

Component	FM611-01 (96 T)	FM611-02 (480 T)
10×Reaction Buffer	1 ml	5 ml
RNase Substrate	1 ml	5 × 1 ml
Positive RNase A（30 pg/μl）	100 μl	500 μl
Nuclease-free Water	5 ml	25 ml

Procedures

1. Preparation before the experiment

The clean bench must be disinfected for 30 minutes. The pipettes, pipette tips and EP tubes used must be nuclease-free to prevent the introduction of exogenous RNase from affecting the experimental results.

2. Sample preparation

Take out the *TransDetect*® Fluorescence RNase Detection Kit from the refrigerator and fully thaw the 10 × Reaction Buffer, RNase Substrate, and Nuclease-free Water at room temperature.

① If the sample is liquid: add the sample directly, or dilute it with Nuclease-free Water to a suitable concentration before use (refer to "Not applicable range" when the concentration range is uncertain for the first test);

② If the sample is solid: dissolve it with Nuclease-free Water to a suitable concentration before use;

③ If the sample is a solid surface: treat the solid surface with Nuclease-free Water before use;

3. System preparation:

3.1 Add 10 μl of RNase Substrate solution into the centrifuge tube, requires protection from light.

3.2 Add 10 μl of 10 × Reaction Buffer into the centrifuge tube containing the substrate solution.

3.3 Adjust the volume of the sample to 80 μl with water and add it into the centrifuge tube. The number of replicates for each sample to be tested and negative control should be no less than 2, and at least one positive control. Add samples according to Table 1 and mix by shaking and centrifugation.

Table 1 Sample reaction system

	Component	RNase Substrate (μl)	10 × Reaction Buffer (μl)	Positive RNase A(μl)	Sample	Nuclease-free Water (μl)	Total Volume (μl)
1	Negative control	10	10	-	-	80	100
2	Negative control	10	10	-	-	80	100
3	Positive control	10	10	5	-	75	100
4	Sample 1	10	10	-	80		100
5	Sample 1	10	10	-	80		100

4. Detection:

4.1 Endpoint fluorescence detection:

① Taking the Invitrogen Qubit 4 Fluorometer as an example: After incubation at 37°C for 1 hour, place the negative control, positive control, and sample tubes into the Invitrogen Qubit 4 Fluorometer, select "Fluorometer" to enter the fluorescence detection interface, select "Blue" for detection, and record the fluorescence value RFU.

② Taking the microplate reader as an example: After incubation at 37°C for 1 hour, set the fluorescence parameters: 37°C, end point fluorescence mode, excitation wavelength is 485 nm, emission wavelength is 528 nm, the gain value needs to be adjusted according to the value of the positive control.

③ Taking qPCR instrument as an example: After incubation at 37°C for 1 hour, 37°C for 30 seconds (collecting fluorescence), 1 cycle, 100 μl reaction volume, and select FAM channel for fluorescence collection.

4.2 Real-time fluorescence detection:

If RNase activity data in real-time is required, the microplate reader or qPCR instrument can be used for detection.

① Taking the microplate reader as an example: Set the fluorescence parameters: shake the plate for 5 seconds at 37°C before detection, read the value every 1 minute for 1 hour, start the kinetic mode, excitation wavelength is 485 nm, emission wavelength is 528 nm, the gain value needs to be adjusted according to the value of the positive control.

② Taking qPCR instrument as an example: 37°C for 1 minute (fluorescence collection), 50 cycles (total time 1 hour), 100 μl reaction volume, and select FAM channel for fluorescence collection.

Interpretation of results

Positive control: the average RFU value of positive control/the average RFU value of negative control ≥ 20 times;

Sample: If the average RFU value of sample/the average RFU value of negative control ≥ 2, it is judged that the sample is contaminated by RNase;

Additional steps: Quantitative detection of RNase A

2-fold serial dilution of Positive RNase A, the concentration of 0.03 pg/μl of RNase A can be detected, the limit of detection is 0.3 pg, and linear relationship within the dilution range ($R^2 > 0.99$) is shown in Figure 1.

1. Preparation of 0.1 × Reaction Buffer. Add 10 μl of 10 × Reaction Buffer to 990 μl Nuclease-free Water, mix well.

2. Dilute RNase A with 0.1 × Reaction Buffer to get different concentrations of RNase A: 1.92 pg/μl, 0.96 pg/μl, 0.48 pg/μl, 0.24 pg/μl, 0.12 pg/μl, 0.06 pg/μl, 0.03 pg/μl, 0.15 pg/μl.

3. Preparation of reaction system according to the table below

Table 2 RNase A quantitative detection reaction system

Reagents	Volume
10 × Reaction Buffer	10 μl
RNase Substrate	10 μl
RNase A	10 μl
Nuclease-free Water	70 μl

4. Detection

Take Invitrogen Qubit 4 Fluorometer as an example. After incubation at 37℃ for 1 hour, select "Fluorometer" to enter the fluorescence detection interface, select "Blue" for detection and record the fluorescence value RFU. The following data is for reference only.

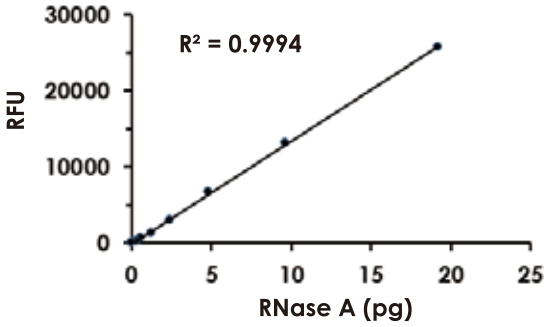

Figure 1. RNase A standard curve

Notes:

1. The maximum excitation wavelength of the fluorophore is 490 nm, and the maximum emission wavelength is 520 nm.
2. To minimize cross-contamination, after adding the positive control RNase A, the waste pipette tips and trash cans should be disinfected immediately.
3. For the sample to be tested, if the concentration of salt ion or the inhibitor is too high, it may lead to a negative result. It is necessary to dilute sample and retest it.
4. Determine whether the sample is compatible with this kit: After the incubation, if no fluorescence appears, add 5 μl positive control RNase A to the sample for reaction, repeat the incubation and fluorescence detection. If strong fluorescence appears, it means that the sample has no interference and is compatible with this kit; If there is still no fluorescence, it means that there are interfering substances in the sample and do not use this kit.
5. If the sample contains substances that can denature proteins (inactivate RNase) or break RNA chains (degrade RNA probe), it will cause false negatives or false positives and not compatible with this kit.