resDetect™ RNase Activity Assay Kit (Fluorescence)

Cat. No. ASE-A001 **Size: 96 tests / 480 tests**

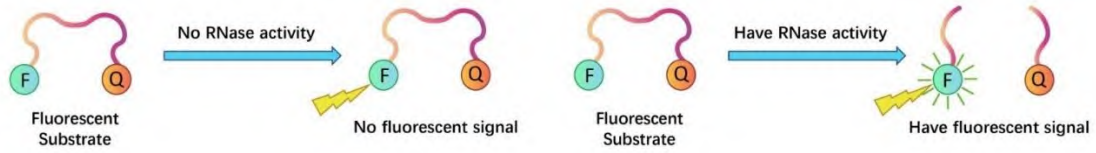
Background

RNases are a class of hydrolytic enzymes that catalyzes both the in vivo and in vitro degradation of ribonucleic acid (RNA) molecules into smaller components. RNase enzymes are categorized into two groups: exoribonucleases and endoribonucleases.

RNases are ubiquitous in the environment, and in some biological materials, they are present in relatively high concentrations. RNases also frequently contaminate common molecular biological reagents such as reaction buffers, enzymes such as reverse transcriptase and RNA polymerase, and buffers for RNA purification and storage. It is often removed by DEPC or heaten from containers in experiments. Since even only minute amounts of RNase contamination would ruin the experiment, it is necessary to evaluate the presence of RNase with the resDetect™ RNase Activity Assay Kit (Fluorescence).

Detection Principle

The resDetect™ RNase Activity Assay Kit (Fluorescence) is based on a fluorophore-labeled RNA substrate. When the sample does not contain RNase activity, the substrate is stable and does not produce a fluorescent signal; when the sample contains RNase activity, the substrate is degraded, resulting in a gradual enhanced fluorescence signal, the rate of increase in fluorescence signal is positively correlated with the dosage and activity of enzymes. Use a fluorescence microplate reader to measure at the wavelength of ex/em = 490/520 nm to determine whether the sample is contaminated by RNase. The rate of fluorescence increase is proportional to the amount and activity of contaminating RNases.



Applications

The resDetect™ RNase Activity Assay Kit (Fluorescence) is a convenient and sensitive assay tool to test the presence of RNase in buffers, reagents, and other components.

Kit Composition

ID	Items	96 Tests	480 Tests	Storage
ASE1-C01	RNase Substrate	2 nmol	10 nmol	-20°C, avoid light
ASE1-C02	10X Reaction Buffer for RNase	10 mL	10 mL	-20°C
ASE1-C03	RNase A (10 μg/mL)	100 μL	500 μL	-20°C
ASE1-C04	TE Buffer (pH 7.0)	1.5 mL	6 mL	-20°C
ASE1-C05	Nuclease-free Water	10 mL	50 mL	-20°C

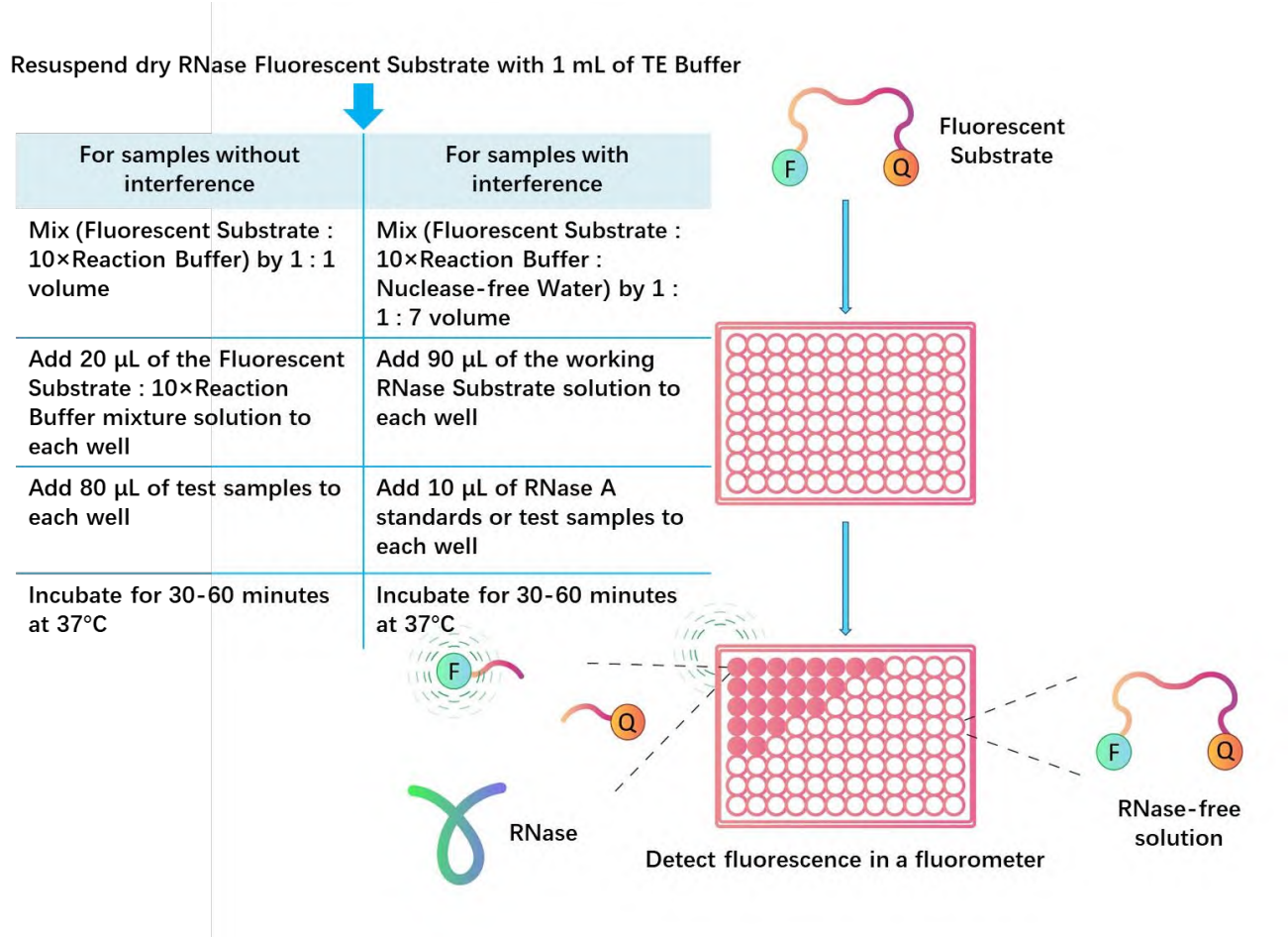
Materials required but not provided

Items	Specifications	Recommended Source
Nuclease-free pipettors, and tips	Nuclease-free	For example, pipettors, and tips from RAININ
Nuclease-free black 96-well plates	Nuclease-free, black non-transparent 96-well plates typically give the lowest background signal	For example, Corning 96 Well Black plates (Cat. No. 3924)
Nuclease-free EP tube	Nuclease-free	-
96-well fluorescence plate reader	Plate reader capable of measuring two or more fluorescent wavelengths in kinetic mode	For example, BMG CLARIOstar Plus Multi-Mode Microplate Reader

Shipping and Storage

1. The product is shipped at dry ice conditions.
2. The unopened kit is stable for 12 months from the date of manufacture if stored at -25~-15℃.
3. The opened kit is stable for up to 3 months from the date of opening at -25~-15℃.
4. If the reconstructed RNase Substrate Solution can't be used out at once, store it at -25~-15°C to avoid repeated freezing and thawing more than 3 times.
5. Do not use reagents past their expiration date.

Quickguide



Preparation before experiment

1. Experimental environment preparation: In order to ensure the accuracy of the experiment, the experimental environment requires that the operation process does not introduce additional RNase. Before you begin, the ultraviolet disinfection of laboratory for 30 minutes is required, and the experimental operation should be carried out in a clean bench (ISO5), clean the operation surface of the clean bench, and switch on the clean bench for ultraviolet irradiation for no less than 30 minutes.

2. Clean equipment surface, if a fluorometer will be used, turn it on and set the following parameters:

Mode	**Kinetic mode using a 96-well plate (if available)**
Excitation/emission (ex/em) maxima	490/520nm
Gain	Set the gain to autoscale if possible. Alternatively use a medium gain setting initially. Note: the setting method of different instruments is not consistent, please consult the instrument supplier for details.
Data collection	Intermittent, 1–1.5 min increments. Use intermittent data collection to limit photobleaching.
Temperature	37°C

3. Materials Preparation: Prepare materials and tools for your experiment, such as Nuclease-free pipettors, and tips, black 96-well plates, EP tubes, the details could refer to "***Materials required but not provided***" in page 2.

4. Reagent Preparation: Take out the kit, equilibrate all the buffer components and RNase A standard to room temperature, make sure that all solution (10×Reaction Buffer, TE Buffer, Nuclease-free Water and RNase A standard) are fully thawed and evenly mixed.

5. RNase Substrate Solution (2 nmol/mL) Preparation: Resuspend 1 tube of lyophilized RNase Substrate with 1 mL of the supplied TE Buffer (pH 7.0), leave it on the ice box for 30 minutes, dissolve the RNA Substrate completely. If the RNase Substrate Solution can't be used out at once, store it at -25~-15°C to avoid repeated freezing and thawing more than 3 times.

Procedure for assay

1. **Prepare 1×Reaction Buffer** by diluting the 10×Reaction Buffer with Nuclease-free Water.
   Calculate the required 1×Reaction Buffer volume, for example, when 1 mL of 1×Reaction Buffer is required, add 0.1 mL of 10×Reaction Buffer into 0.9 mL of Nuclease-free Water.
2. **Prepare the samples**
   The recommended sample volume is 10 µL or 80 µL per well. The volume of sample addition can be determined according to the sample type and the presence or absence of interference.
   1) When measuring water samples such as process water, injection water, etc., in the absence of interference, it can add 80 µL of water samples.
   2) When test solid surfaces such as pipette tips, pH electrodes, glass beads and so on, it is recommended to add 80 µL samples, if the test sample are pipette tips, please soak the pipette tips in nuclease-free water 10 times and immersed in water for a few minutes. If the sample is other solid that cannot be directly immersed in nuclease-free water, wipe the solid surface with a nuclease-free cotton swab and immerse the swab in the nuclease-free water for a few minutes. Collect the solution, the volume of solution should be more than 80µL, and the solution usually does not contain interfering substances, if the volume of the test sample is less than 80 µL, the sample can be diluted to 80 µL with nuclease-free water provided by the kit.
   When the sample volume is 80 µL, the test can be carried out according to the following method 1, the detailed preparation process of the RNase Substrate Working Solution, please refer to "4. Prepare the RNase

Substrate Working Solution":

Mix the 2nmol/mL RNase Fluorescent Substrate and 10×Reaction Buffer by 1:1 volume, add 20 µL of the mixture solution to each 96-well plate, and add 80 µL of test samples to each well, incubate for 30 minutes to 1 hour at 37°C.

3) All samples with a concentration above the highest standard (Std 7) must be diluted in 1×Reaction Buffer. Because nuclease activity is greatly affected by pH and salt, you need to know the exact composition of your samples and solution incompatibility, some samples may need to be diluted to avoid interference, the specific requirements for samples and solutions could refer to "***Frequently asked questions (FAQs)***" in page 11.

If your sample is a lyophilized powder, and needs to be reconstructed, please reconstitute the sample following the CoA, and note that the solution or water used for reconstitution MUST be nuclease-free.

When test the samples with interference or the samples needs to be diluted, it is recommended to add 10uL of samples to each well, dilute the samples with 1×Reaction Buffer, detect the samples as follow method 2, the detailed preparation process of the RNase Substrate Working Solution, please refer to "4. Prepare the RNase Substrate Working Solution":

Prepare the RNase Substrate working solution by add equal volume of 2nmol/mL RNase Substrate and 10×Reaction Buffer into 7 times the volume of Nuclease-free Water (for example, add 10 µL of 2nmol/mL RNase Substrate and 10 µL of 10×Reaction Buffer into 70 µL of nuclease-free Water, up to 90 µL of the working RNase Substrate solution to be tested). Add 90 µL of the working RNase Substrate solution to each 96-well plate, and add 10 µL of RNase A standards or test samples, incubate for 30 minutes to 1 hour at 37°C.

Sample Type	**Recommended adding volume of samples**	**sample treatment**	**RNase Substrate Working Solution**
Water	80 µL	Direct addition	Add 20 µL of the Fluorescent Substrate: 10×Reaction Buffer mixture solution (1:1 volume) to each well
Solid surfaces	80 µL	Treat the solid surfaces with nuclease-free water and then test	
Samples with interference or needs to be diluted	10 µL	Dilute the samples with 1×Reaction Buffer and then test	Add 90 µL of the working RNase Substrate solution to each well

Note: *The recovery rate of each testing sample shall be determined, and the recovery rate should be within a reasonable range (such as 80% ~ 120%), please refer to the "Frequently asked questions (FAQs)" in page 11 for detailed recovery determination procedure.*

3. Prepare the working RNase A standards

Each well requires 10 µL of standard according to the method, it is recommended that the number of wells for standards should be not less than 2.

Serially dilute the **RNase A** standard stock solution with the 1×Reaction Buffer to prepare standards. To avoid introducing extra RNase A, all tips and EP tubes and other consumables should be Nuclease-free, and all buffers also should be Nuclease-free. In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended RNase A standard dilution procedure is listed and illustrated below (1×Reaction Buffer is recommended as the Sample Dilution Buffer):

1) Thaw the RNase A standard stock solution, the original concentration of the RNase A standard stock solution is 10 µg/mL.

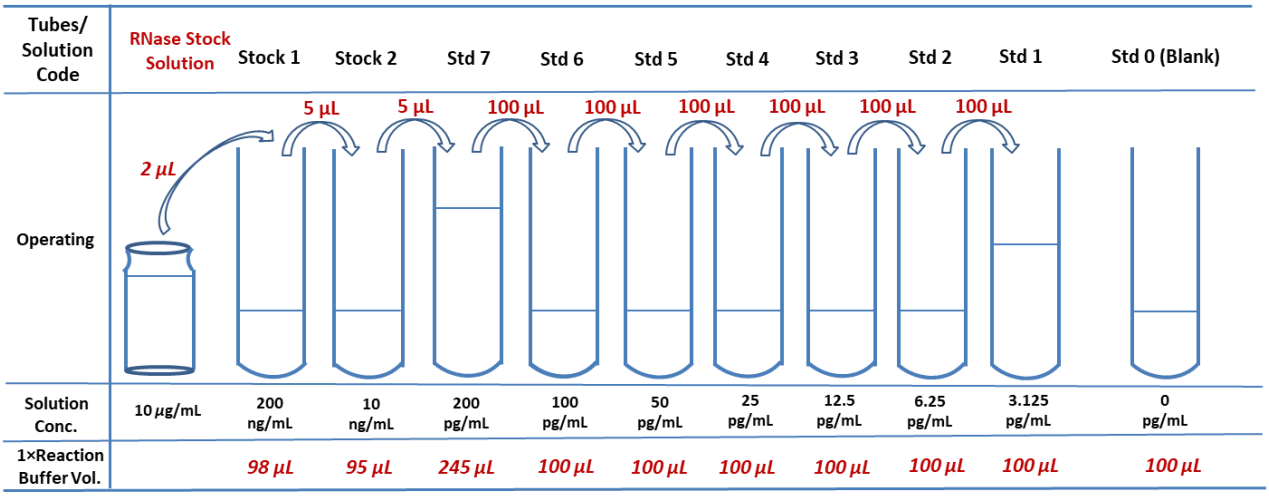
2) Prepare Stock1 by diluting the 10 µg/mL of standard stock solution 50-fold with 1×Reaction Buffer to 200 ng/mL (Stock1): Dilute 2 µL RNase A stock solution into 98 µL 1×Reaction Buffer, mix gently well.

3) Then prepare Stock2 by diluting the 200 ng/mL of standard stock solution (Stock1) 20-fold with 1×Reaction Buffer to 10 ng/mL(Stock2): Dilute 5 µL Stock1 into 95 µL 1×Reaction Buffer, mix gently well.

4) Prepare the highest concentration of standard curve Std 7 (200 pg/mL) by diluting the 10 ng/mL of standard stock solution (Stock2) 50-fold with 1×Reaction Buffer to 200 pg/mL: Dilute 5 µL Stock2 into 245 µL 1×Reaction Buffer.

5) At last, prepare 1:1 serial dilutions for the standard curve as follows (***take 200 µL of each concentration of standards as example***):

- Pipette 100 µL of 1×Reaction Buffer into each vial from Std6 to Std1;
- Add 100 µL of RNase A standard 7 to 100 µL of 1×Reaction Buffer, mix gently and repeat the serial dilution to make RNase A standard solutions: std6, std5, std4, std3, std2, std1, make sure to mix well every time, this will create 7 standards for the analyte;
- Std0 (Blank) is 1×Reaction Buffer alone.



Standard	Diluent Ratio	Serial Dilutions	Concentration	Weight per well
Stock solution1 (Stock1)	50	2 µL 10µg/mL stock solution + 98 µL 1×Reaction Buffer	200 ng/mL	/
Stock solution2 (Stock2)	20	5 µL Stock1 + 95 µL 1×Reaction Buffer	10 ng/mL	/
Standard 7	50	5 µL Stock2 + 245 µL 1×Reaction Buffer	200 pg/mL	2 pg
Standard 6	2	100 µL Standard 7 + 100 µL 1×Reaction Buffer	100 pg/mL	1 pg
Standard 5	2	100 µL Standard 6 + 100 µL 1×Reaction Buffer	50 pg/mL	0.5 pg
Standard 4	2	100 µL Standard 5 + 100 µL 1×Reaction Buffer	25 pg/mL	0.25 pg
Standard 3	2	100 µL Standard 4 + 100 µL 1×Reaction Buffer	12.5 pg/mL	0.125 pg
Standard 2	2	100 µL Standard 3 + 100 µL 1×Reaction Buffer	6.25 pg/mL	0.0625 pg
Standard 1	2	100 µL Standard 2 + 100 µL 1×Reaction Buffer	3.125 pg/mL	0.03125 pg
Standard 0	-	100 µL 1×Reaction Buffer	0 pg/mL	0 pg

4. **Prepare the RNase Substrate working solution**

1) When the required volume of sample is 80 µL, each well requires 10 µL of RNase Substrate stock solution and 10 µL of 10×Reaction Buffer, totally 100 µL per well. Mix the RNase Substrate and 10×Reaction Buffer by 1:1 volume to get the mixture solution **(RNase Substrate Working Solution)**, and add 20 µL of **RNase Substrate Working Solution** to each well. For example, when 50 wells are required for experiment, 1 mL of

RNase Substrate Working Solution is required, we recommend to prepare 1.1 mL of RNase Substrate Working Solution, that is, mix 550 μL of 2 nmol/mL RNase Substrate with 550 μL of 10×Reaction Buffer, totally up to 1.1 mL of the working RNase Substrate solution required for following experiment.

Please refer to the following methods to prepare the **RNase Substrate Working Solution**:

Tests	Each Well Volume	RNase Substrate	10×Reaction Buffer	RNase Substrate Working Solution Volume
50 Tests	20 μL	550 μL	550 μL	1100 μL

2) When the required volume of sample or standards is 10 μL, each well requires 90 μL of RNase Substrate working solution. Calculate the required total volume of RNase Substrate working solution according to the wells number in the experiment. Add equal volume of 2 nmol/mL RNase Substrate and 10×Reaction Buffer into 7 times the volume of Nuclease-free Water. For example, When the number of experimental wells is 50, 4.5 mL of RNase Substrate working solution is required, we can prepare 4.95 mL of RNase Substrate working solution to ensure a margin, add 550 μL of 2 nmol/mL RNase Substrate and 550 μL of 10×Reaction Buffer into 3.85 mL of Nuclease-free Water, up to 4.95 mL of the working RNase Substrate solution to be tested.

Please refer to the following methods to prepare the solution:

Tests	Working solution	RNase Substrate	10×Reaction Buffer	Nuclease-free Water
50 Tests	4950 μL	550 μL	550 μL	3850 μL

5. **Add the above prepared RNase Substrate Working Solution to each well, then RNase A standards and samples, mix on plate shaker at 500 rpm for 5~10 seconds, and incubate 30-60 min under 37 degree, finally read the fluorescence signal value.**

Standards and samples can be added as shown below:

It is recommended that each concentration of standards and your samples be reperforated. If you need to add your own positive references and negative references, references can be added according to the requirements of your own enterprise standards, usually the number of wells for the positive references should be not less than 1, and the number of wells for the negative references should be not less than 2:

1) If you have access to a fluorometer capable of collecting data in real-time:

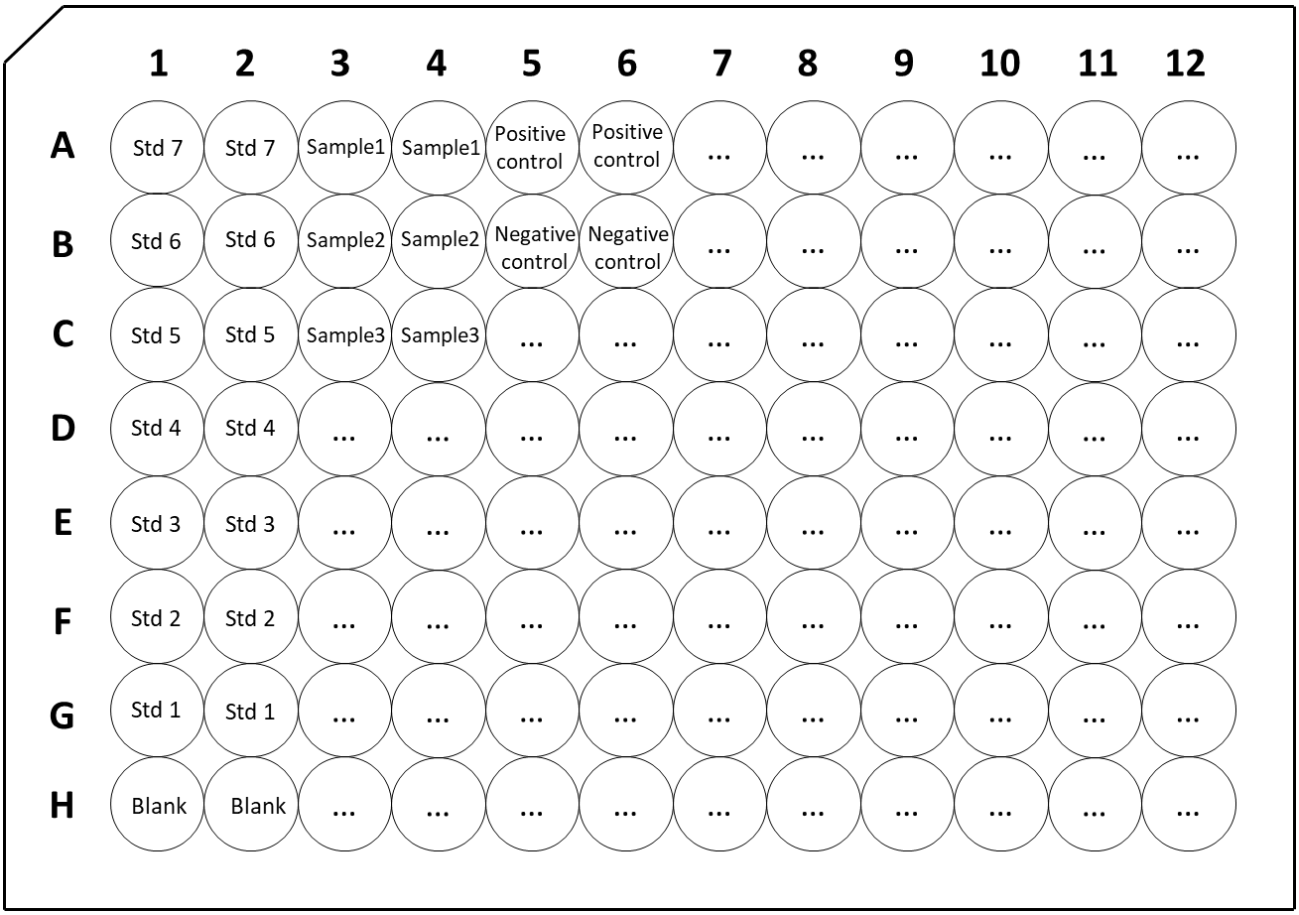
Add the corresponding volume of working RNase Substrate solution, RNase A standards or samples to each 96-well plate, incubate the plate in the fluorometer collecting real-time data at 1~1.5 minutes intervals for 30~60 minutes at 37°C using the settings described in this section. The RNase Activity Assay can be evaluated in rigorous kinetic terms. Using real-time data, RNase activities can be compared using enzyme velocity measurements.

2) If you do not need real-time data from the RNase Activity Assay, you can measure the fluorescence signals by endpoint measurement using a fluorometer.

Add the corresponding volume of working RNase Substrate solution, RNase A standards or samples to each 96-well plate, after incubate for 30~60 minutes at 37°C, measure the sample fluorescence using the settings described in this section.

Note: *The RNase standards and all test samples should be measured at the same gain setting for the same plate.*

Plate Layout



Data processing & Interpretation

Sample Type	Expected Result
RNase Standards	All 7 RNase Standards were positive, take RFU (std 0) and RFU (standard) as the ordinate and RNase concentration of standard as the abscissa, fitting the standard curve with four parameters logistic model, and the correlation coefficient R^2 should be ⩾ 0.99.
Positive References	The signals of Positive references should be positive, and the concentration calculated back according to the standard curve should conform to the nominal concentration.
Negative references	The negative references have a minimal fluorescence (background). And the value is basically close to blank control value.
Test samples	If RFU of sample ⩾ 2×RFU of blank, it is considered that the sample to be tested is contaminated by RNase. If the sample contains interfering substances, it may result in false negative results, at this time, the sample to be tested shall be pre diluted with nuclease-free water, and then tested.
Blank	The signals of blank (std 0) is the background value of the experiment should be as low as possible, the background value is different because of different fluorometer. All other samples are judged against this value.

Typical Data

Real-time fluorescent monitoring of RNase A activity in RNase Activity Assay Kit:

Add 90 µL RNase Substrate Working Solution (mix RNase Substrate, 10×Reaction Buffer and Nuclease-free Water by 1:1:7 volume) to each 96-well plate, and add 10 µL RNase A standards (0-200 pg/mL×10 µL / well = 0-2 pg/well), incubate the plate in the fluorometer (BMG CLARIOstar) collecting real-time data at one minute intervals for 30 minutes at 37°C using the settings described in this section. The RNase Activity Assay can be evaluated in rigorous kinetic terms using real-time data.

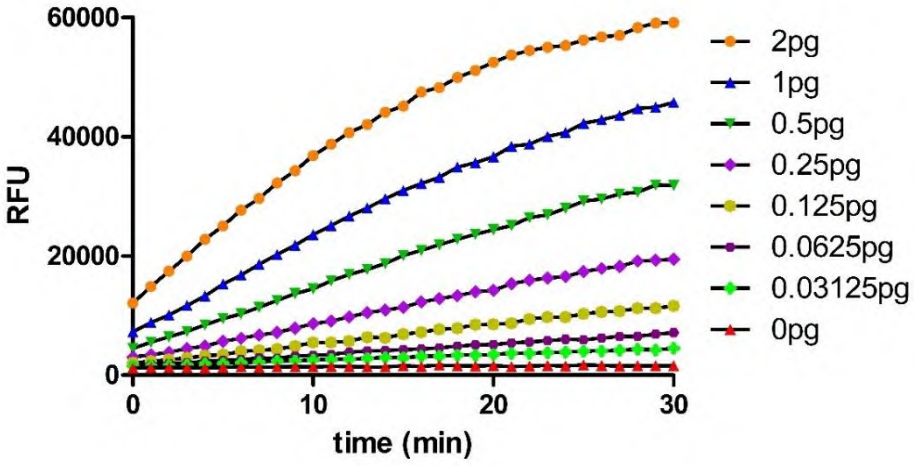


Figure1 Real-time fluorescent monitoring of RNase A activity

Standard curve of resDetect™ RNase Activity Assay Kit (Fluorescence):

This assay kit employs a standard detection of RNase A. Add 90 µL RNase Substrate Working Solution (mix RNase Substrate, 10×Reaction Buffer and Nuclease-free Water by 1:1:7 volume) to each 96-well plate, and add 10 µL of RNase A standards (0-200 pg/mL×10 µL/well = 0-2 pg/well), incubate for 30 minutes at 37°C. Then measure the fluorescence using the settings described in this section in a fluorometer **(BMG CLARIOstar)**. Take RFU of standards as the ordinate and RNase concentration as the abscissa. Four parameters logistic are used to draw the standard curve. This following data is for reference only.

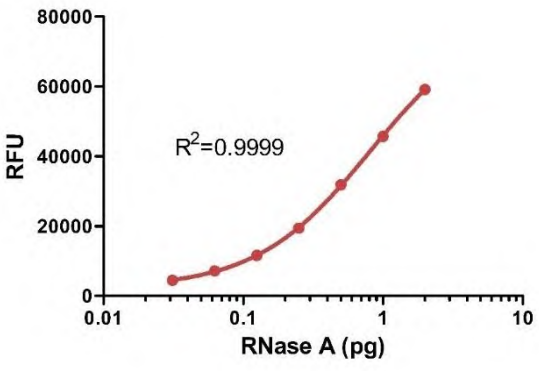


Figure2 Standard curve of RNase Activity

Std. weight (pg/well)	RFU-1	RFU-2	Ave (RFU)
0 pg	1560	1700	1630
0.03125 pg	4507	4172	4340
0.0625 pg	7145	6390	6768
0.125 pg	11640	10428	11034
0.25 pg	19411	18100	18756
0.5 pg	31812	28705	30259
1 pg	45714	43370	44542
2 pg	59114	56879	57997

Sensitivity

Assay range (pg/well)	Limit of quantification (LoQ*)
0.03125-2 pg/well	0.03125 pg

Intra-Assay Statistics

Sample	1	2	3	4	5	6	7
Number of Replicate	8	8	8	8	8	8	8
Mean RFU	3506	5964	9831	16862	27758	41779	56643
Standard Deviation	130	192	291	532	180	783	412
Coefficient of Variation (%)	3.7	3.4	3.0	3.2	0.6	1.9	0.7

Inter-Assay Statistics

Sample	1	2	3	4	5	6	7
Number of Replicate	8	8	8	8	8	8	8
Mean RFU	3858	6132	10503	17609	28071	41161	53533
Standard Deviation	579	769	1495	2307	3341	4203	4249
Coefficient of Variation (%)	15	12.5	14.2	13.1	11.9	10.2	7.9

Recovery

	System	5.5 μg/mL of Pyrophosphatase (n=2)		1% of (50mM Tris-HCl and 50% Glycerol) (n=2)		2 μg/mL Thermostable Inorganic Pyrophosphatase (n=2)		1×Reaction Buffer (n=2)	
Sample	weight. (pg)	Calculated weight. (pg)	Ave % RE	Calculated weight. (pg)	Ave % RE	Calculated weight. (pg)	Ave % RE	Calculated weight. (pg)	Ave % RE
Sample 1	1.5	1.4252	95	1.3319	89	1.3375	89	1.4193	95
Sample 2	0.2	0.1691	85	0.1669	83	0.1683	84	0.1839	92
Sample 3	0.05	0.0437	87	0.0461	92	0.0442	88	0.0472	94

Note： *All solution concentrations correspond to the final concentration in the 100uL reaction system.*

Frequently asked questions (FAQs)

1. Which enzymes can this kit detect except for RNase A?

In addition to RNase A, the resDetect™ RNase Activity Assay Kit (Fluorescence) is also optimized for the detection of RNase T1, RNase 1 and micrococcal nuclease, Benzonase nuclease, mung bean nuclease, and S1 nuclease.

2. Which solutions are incompatible?

Most reaction buffers and solutions that are used with RNA can be tested with resDetect™ RNase Activity Assay Kit (Fluorescence). Below are listed the types of solutions that are not compatible with kit:

Incompatible solutions	Notes
Gel loading buffers and other darkly colored solutions	Darkly colored solutions may interfere with excitation of the fluorophore or may block its light emission, making them incompatible with resDetect™ RNase Activity Assay Kit (Fluorescence).
Solutions that inhibit RNase activity	The following solutions are known to inhibit RNase: • Solutions with high ionic strength (e.g. 5 M NaCl, 20X SSC, 3 M sodium acetate, etc.) • Solutions with pH <4 or >9 • Chaotropic agents, detergents, chelating agents, or any solutions that denature proteins (e.g. SDS, guanidine thiocyanate, urea, EDTA, etc.)
Solutions that cause chemical instability of the resDetect™ RNase Activity Assay Kit (Fluorescence)	Solutions that chemically degrade the substrate may produce false positive signals. The RNase Substrate is unstable in the following types of solutions: • Solutions with pH >9 • Caustic solutions (strong acids and bases, bleach)

3. How to determine solution compatibility?

1) Test the solution following the standard procedure.

2) At the end of the incubation, if no fluorescence above the minus-RNase control is seen, add 5 µL of the supplied RNase A to the completed reaction, and repeat the incubation and signal detection. Compatible solutions will strongly fluoresce after incubation with RNase A.

If your sample solutions do interfere with the kit assay, it is recommended to dilute your sample with 1×Reaction Buffer before introduction of sample into the wells. Minimal dilution factor needed to be verified to avoid any interference.

4. How to determine the sample recovery rate?

The recovery rate of each testing sample shall be determined, and the recovery rate should be within a reasonable range (such as 80% ~ 120%), the detailed recovery determination procedure is as follows:

1) This recovery test experiment can be performed by spiking a RNase standard provided with this kit with concentration in the linear range into the testing samples, the total amount of added RNase and endogenous RNase from the sample itself should not exceed the highest standard (Std 7), that means the total amount of RNase should not exceed 2pg per well. All samples with a concentration of RNase above the highest standard (Std 7) must be diluted to a reasonable concentration, then the standard is added for recovery testing, or your

sample contains interfering ingredients, it also needs to be diluted to reduce interference.

2) When the required sample volume is 80 μL, if the volume of the test sample is less than 80 μL, the sample can be supplemented to more than 80 μL with nuclease-free water provided by the kit, then add a known concentration of RNase standard into sample according to the 1:7 volume ratio. Since the total amount of RNase should be in the linear range(⩽2pg per well), it is recommended that the final total concentration of added RNase and endogenous RNase from the sample itself should not exceed 25pg/mL. For example, adding 1 part of the 25 pg/mL RNase standard to 7 part of test sample, and then add 80 μL of the mixture per well, it is equivalent to adding 0.25pg of RNase standard per well.

   First, determine the RNase amount (M1) of the samples itself by adding 1 part of the1×Reaction Buffer to 7 part of test sample, and then add 80 μL of the diluted test sample per well. Next add a known amount of RNase standard (M2) to test sample by 1:7 volume ratio, and determine the total RNase amount (M3) of the mixed sample/standard, the recovery calculation formula is as follows: (M3-M1) / M2 × 100%, the experimental design is as follows:

Sample Recovery ID	**Mixed Ratio of Standard/Buffer: Sample**	**Adding components and volumes per well**	**Adding Volume of sample per well**	**Endogenous RNase from the sample itself per well**	**Adding amount of RNase Standard per well**	**Total amount of RNase per well**
Sample	1:7	1×Reaction Buffer, 10 μL	70 μL	M1	0 pg	M1
Sample-R	1:7	Standard 4 (25 pg/mL) 10 μL	70 μL	M1	M2 = 0.25 pg	M3

3) When the required sample volume is 10 μL, if the sample needs to be diluted, dilute the sample with 1×Reaction Buffer, then add a known concentration of RNase standard into sample according to the 1:1 volume ratio. Since the total amount of RNase should be in the linear range(⩽2pg per well), it is recommended that the final total concentration of added RNase and endogenous RNase from the sample itself should not exceed 200pg/mL. For example, adding 1 part of the 50 pg/mL RNase standard to 1 part of test sample will yield an additional spike of 25pg/mL. and then add 10 μL of the mixture per well, it is equivalent to adding 0.25pg of RNase standard per well.

   Any endogenous RNase from the sample itself determined prior to spiking and corrected for by the 50% dilution of that sample should be subtracted from the value determined for the spiked sample, then calculated the concentration of RNase to give the recovery rate.

   For example, to determine the standard recovery of diluted samples, it is necessary to first determine the RNase amount (M1) of the samples itself by adding 1 part of the1×Reaction Buffer to 1 part of test sample, and then add 10 μL of the diluted test sample per well. Next add a known amount of RNase standard (M2) to test sample

by 1:1 volume ratio, and determine the total RNase amount (M3) of the mixed sample/standard, the recovery calculation formula is as follows: (M3-M1) / M2 × 100%, the experimental design is as follows:

Sample Recovery ID	Mixed Ratio of Standard/Buffer: Sample	Added components and volumes per well	Adding Volume of sample per well	Endogenous RNase from the sample itself per well	Adding amount of RNase Standard per well	Total amount of RNase per well
Sample	1:1	1×Reaction Buffer, 5 µL	5 µL	M1	0 pg	M1
Sample-R	1:1	Standard 5 (50 pg/mL), 5 µL	5 µL	M1	M2 = 0.25 pg	M3

5. How to test solid surfaces?

Pipette tips, pH electrodes, glass beads and other solid surfaces can be tested for RNase by preparing a mock RNase reaction as described for the minus-RNase control. Immerse the object in the reaction mixture for a few minutes (pipet up and down for pipette tips), and then check the solution for fluorescence as described in the protocol.

6. What should be done when suspected false positive or false negative results appearing?

1) In case of false positive in the experiment, firstly, the experimental consumables are excluded from nuclease contamination. Secondly, the experimental solution is inspected to ensure that all solutions are free of nuclease contamination and have no degradation effect on the RNase Substrate. At last, make sure there is no additional nuclease introduced by error operations.
2) When the experiment has false negative results, check whether the solution has RNase activity inhibitor or fluorophore blocking component. An inappropriate solution will produce false negative results.