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ab273299
RNase Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273299
(use https://www.abcam.cn/ab273299 for china, or
https://www.abcam.co.jp/ab273299 for Japan)

For the determination of RNase activity in buffers, reagents, and other components as well as quantitatively evaluate RNase activity of recombinant enzymes in real time.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

RNase Activity Assay Kit (Fluorometric) (ab273299) enables researchers to measure RNase activity in buffers, reagents, and other components as well as quantitatively evaluate RNase activity of recombinant enzymes in real time.

The assay uses a highly sensitive, specific probe that releases a fluorescent product in the presence of active RNase. The limit of detection is 0.4 pg RNase/well and limit of quantification is 1.2 pg RNase/well.

2. Protocol Summary

Prepare Samples, Positive Control and Negative Control as directed

Prepare standard curve as directed

Prepare Reaction Mix as directed

Add Positive Control, Samples and Negative Control to appropriate wells and adjust volume to 50 µL

Add Reaction Mix (10 µL) to samples, Positive Control and Negative Control wells

Measure fluorescence (Ex/Em = 495/520 nm)

RNase contamination detection:

Measure initial (t = 0 hrs) fluorescence and at the desired incubation time (1-3 hr).

Activity quantification:

Measure fluorescence in kinetic mode every 2 minutes for at least 30 - 60 minutes at 25˚C.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
10X RNase Buffer	1 mL	-20°C	RT
RNase Probe	1 vial	-20°C	-20°C
Molecular Biology (RNA) Grade Water	25 mL	-20°C	RT
96-Well Half Area Plate (with lid)	1 Unit	-20°C	RT
RNase Positive Control	250 µL	-20°C	-20°C
Fluorescence Standard I	100 µL	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Spectrophotometer;
- RNase-free barrier pipette tips;
- Certified RNase-free reagents, buffers.

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 10X RNase Buffer:

Ready to use. Store at room temperature.

9.2 RNase Probe:

Keep on ice while in use.
Reconstitute with 110 µL Molecular Biology (RNA) Grade Water.
Keep away from light. Aliquot and store at -20°C. Avoid multiple freeze-thaw cycles.

9.3 Molecular Biology (RNA) Grade Water:

Ready to use. Store at room temperature.

9.4 96-Well Half Area Plate (with lid):

Ready to use. Store at room temperature.

9.5 RNase Positive Control:

Keep on ice while in use. Ready to use. Aliquot and store at -20°C.

9.6 Fluorescence Standard I:

Ready to use. Keep away from light. Store at -20°C.

10. Sample Preparation

Δ Note: For accurate estimations, it is crucial to use molecular biology grade reagents (RNase free) during sample preparation and RNase barrier filter tips for sample pipetting at all times in order to avoid RNase contamination.

10.1 Solutions with suspected RNase contamination:

10.1.1 Add 2-44 µL of solution into 96-Well Half Area Plate (with lid).

Surfaces with suspected RNase contaminations:

10.1.2 Put 100 µL of the Molecular Biology (RNA) Grade Water onto the surface.

10.1.3 Pipette 44 µL of this liquid sample with a pipette and transfer it to a well in the 96-Well Half Area Plate (with lid).

Purified RNase:

10.1.4 Prepare several dilutions with Molecular Biology (RNA) Grade Water to make sure that the kinetic curve falls within the Standard Curve range.

10.1.5 Add 2-44 µL of sample to appropriate wells of 96-Well Half Area Plate (with lid).

Positive Control:

10.1.6 Make a 100-fold dilution of the Positive Control by adding 5 µL of the RNase Positive Control into 445 µL of Molecular Biology (RNA) Grade Water and 50 µL of 10X RNase Buffer.

10.1.7 Make a 10,000-fold dilution of the Positive Control by adding 5 µL of the 100-fold dilution to 495 µL of Molecular Biology (RNA) Grade Water.

10.1.8 Add 2-10 µL of the 10,000-fold dilution into a well of the 96-Well Half Area Plate (with lid).

Negative Control:

10.1.9 Add 44 µL of Molecular Biology (RNA) Grade Water to appropriate wells of the 96-Well Half Area Plate (with lid).

10.2 All Wells:

10.2.1 Bring volume of all wells to 44 µl with Molecular Biology (RNA) Grade Water.

10.2.2 Add 6 µL 10 X RNase Buffer into all wells.

11. Standard Curve

11.1 Prepare a 100 µM solution of Standard solution by diluting 10 µL of 10 mM RNase Standard with 990 µL Molecular Biology (RNA) Grade Water.

11.2 Prepare a 5 µM Standard solution by diluting 10 µL of 100 µM Standard solution into 190 µL of Molecular Biology (RNA) Grade Water.

11.3 Add 0, 2, 4, 6, 8, 10 µL of the 5 µM Standard solution into a series of wells in the 96-Well Half Area Plate (with lid), resulting in 10, 20, 30, 40, 50 pmol RNase Standard per well.

11.4 Adjust well volumes to 54 µL with Molecular Biology (RNA) Grade Water.

11.5 Add 6 µL 10 X RNase Buffer into all Standard wells.

Δ Note: Final volume for all Standard wells should be 60 µL.

Standard #	5 µM RNase-Standard (µL)	Mol.Bio.Grade Water (µL)	10 X RNase Buffer (µL)	RNase (pmol/well)
1	10	44	6	50
2	8	46	6	40
3	6	48	6	30
4	4	50	6	20
5	2	52	6	10
6	0	54	6	0

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

12.1.1 Prepare Samples, Positive Controls and Negative Controls as described in Section 10.

Reaction Mix:

12.1.2 Prepare enough reagents for the number of assays to be performed. For each well, prepare 10 µL of the Reaction Mix:

Component	Volume to add per well (µL)
RNase Probe	1
Molecular Biology (RNA) Grade Water	9

12.1.3 Add 10 µL of Reaction Mix into each of Sample, Positive Control and Negative Control well. Mix well for 30 to 60 seconds.

Δ Note: Do not add Reaction Mix to the Standard wells.

Δ Note: Total volume for all wells = 60 µL.

RNase contamination detection:

12.1.4 Measure initial (t = 0 hrs) fluorescence (Ex/Em = 495/520 nm) in endpoint mode.

12.1.5 Place the cover on the plate and incubate for between 1-3 hours at room temperature avoiding light.

12.1.6 Measure fluorescence at the desired incubation time (1-3 hours).

12.1.7 Make sure that there is no significant increase in negative control by avoiding cross-contamination in the Sample wells.

For activity quantification:

12.1.8 Measure fluorescence (Ex/Em = 495/520 nm) in kinetic mode every 2 minutes for at least 30 - 60 minutes at 25˚C.

Δ Note: Presence of RNase inhibitors or pH can affect the detection of contaminants and cause false negatives. Add 10 µL of the 10,000-fold diluted Positive Control into suspected false negative wells. Incubate in the dark for 10 minutes at room temperature and measure the fluorescence.

Δ Note: Limit of detection of RNase is 0.4 pg/well.

13. Calculations

13.1 RNase contamination can be defined as:

$$RFU_{(t)} \geq 2 \times RFU_{(t=0\ min)}$$

If the fluorescence of the Sample well is 2-fold higher than its initial value (t = 0 h), then RNase activity must be considered. Thus, reagents contaminated with RNase should be discarded in order to maintain RNA-free experiments.

Presence of RNase inhibitors or pH can affect the detection of contaminants and cause false negatives. Add 10 µL of the 10,000-fold diluted Positive Control into suspected false negative wells. Incubate in the dark for 10 minutes at room temperature and measure the fluorescence.

The absence of RNase inhibitors in Samples suspected false negative can be confirmed if:

$$RFU_{(t=10\ min)} \geq 5 \times RFU_{(t=0\ min)}$$

Fluorescence should be at least 5-fold higher when compared to initial reading if RNase inhibitors are absent from the solution.

13.2 Quantification of RNase Activities:

13.2.1 Plot the RNase Probe Standard Curve with pmol of RNA on the x-axis and RFU on the y-axis.

13.2.2 Apply a linear fit to the RNase Standard values and determine the Standard Curve equation.

13.2.3 Samples/Positive Control: Plot RFU on the y-axis vs. time (in minutes) on the x-axis and determine the slope (RFU/min) of the linear portion of the reaction curve.

13.2.4 Apply slope (RFU/min) to the Standard Curve to obtain S (pmol/min).

13.2.5 Estimate RNase kinetic signal by subtracting Negative Control readings (NC) from Samples (S). R =S - NC

13.2.6 Calculate Sample activity as follows:

$$Sample\ RNase\ activity\ = \frac{R}{V}\ x\ D\ (pmol/min/\mu L = \ \mu U/\mu L)$$

$$Sample\ Specific\ activity\ = \frac{R}{V\ x\ P}\ x\ D\ (pmol/min/\mu g = \ \mu U/\mu g)$$

R = Corrected activity (pmol/min).
V = Sample volume added into the reaction well (µL).
D = Dilution Factor.
P = Protein concentration of Sample added into the well (µg/µL).

Unit definition:

One unit of RNase activity is the amount of enzyme that cleaved 1.0 µmol of RNase Probe per minute at 25 °C.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

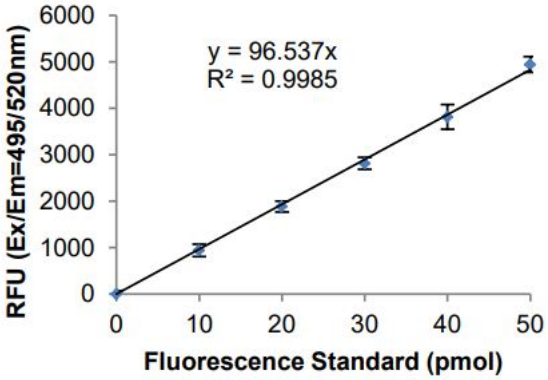

Figure 1. RNase Fluorescence Standard Curve.

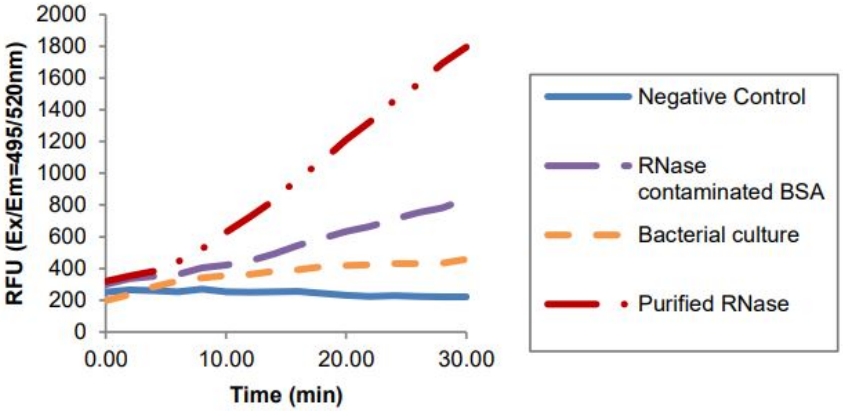

Figure 2. Sample kinetic curve obtained from Negative Control (Blank), bacterial culture, RNase contaminated BSA and purified RNase.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)