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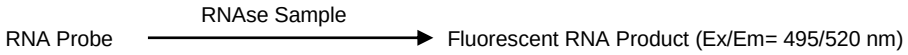
RNase Activity Detection/Quantification Assay Kit (Fluorometric)

02/19

(Catalog # K934-100; 100 assays; Store at -20°C)

I. Introduction:

Many molecular biology applications require RNase-free environment due to the degradation of RNA samples by RNase. Obtaining full length, high quality RNA samples are essential, yet challenging in genomics settings. BioVision's RNAse Activity Detection Assay Kit 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.



II. Applications:

- Measurement of RNAse contamination in buffers and samples
- Quantitative analysis of RNAse activity of purified enzymes

III. Sample Type:

- Buffers, assay reagents, samples
- Purified enzyme

IV. Kit Contents:

Components	K934-100	Cap Code	Part Number
10X RNase Buffer	1 ml	Red	K934-100-1
RNA Probe	1 vial	White	K934-100-2
Molecular Biology Grade Water	25 ml	NM	K934-100-3
Half-area Plate	1 plate	--	K934-100-4
RNase Positive Control	250 µl	Blue	K934-100-5
Fluorescence Standard (10 mM in DMSO)	100 µl	Yellow	K934-100-6

V. User Supplied Reagents and Equipment:

- Spectrophotometer
- RNAse-free barrier pipette tips
- Certified RNAse-free reagents, buffers

VI. Storage Conditions and Reagent Preparation:

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.

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Keep all components on ice while in use. Read entire protocol before performing the assay.

- **10X RNase Buffer, Molecular Biology Grade Water:** Warm to room temperature. Ready to use. Store at RT.
- **RNase Probe:** Reconstitute with 110 µl Molecular Biology Grade Water. Aliquot and store at -20ºC. Avoid multiple freeze-thaw cycles. Keep away from light.
- **Half-area Plate:** Ready to use. Store at RT.
- **RNAse Positive Control:** Ready to use. Aliquot and store at -20°C.
- **Fluorescence Standard (10 mM):** Warm to room temperature. Ready to use. Store at -20°C. Keep away from light.

VII. RNAse Activity Detection Assay Protocol:

1. **Sample Preparation: For solutions with suspected RNase contamination:** Add 2-44 µl of solution into a half-area plate. **For surfaces with suspected RNase contaminations:** Put 100 µl of the Molecular Biology Grade Water onto the surface. Pipette 44 µl of this liquid sample with a pipette and transfer it to a well in the half-area plate. **For purified RNase:** Prepare several dilutions with Molecular Biology Grade Water (provided) to make sure that the kinetic curve fall within the Standard Curve range. Add 2-44 µl of sample to each well in the Half-area Plate. **For Positive Control**: Make a 100-fold dilution of the Positive Control by adding 5 µl of the RNase Positive Control into 445 µl of Molecular Biology Grade Water and 50 µl of 10X RNase Buffer. Make a 10,000-fold dilution of the Positive Control by adding 5 µl of the 100-fold dilution into 495 µl of Molecular Biology Grade Water. Add 2-10 µl of the 10,000-fold dilution into a well of the Half-area Plate. **For all Samples:** Bring volume of all Sample wells to 44 µl with Molecular Biology Grade Water. **Negative Control (NC):** Aliquot 44 µl Molecular Biology Grade Water to a well. Add 6 µl 10 X RNase Buffer into all wells containing Samples, Positive Control and Negative Control. *Partial volume for all Sample wells should be 50 µl.*
2. **RNA Probe Standard Curve:** Make a 100 µM Standard solution by adding 10 µl of 10 mM RNase Standard into 990 µl Molecular Biology Grade Water. Make a 5 µM Standard solution by adding 10 µl of 100 µM Standard solution into 190 µl of Molecular Biology Grade Water. Add 0, 2, 4, 6, 8, 10 µl of the 5 µM Standard solution into a series of wells in the half-area plate, resulting in 10, 20, 30, 40, 50 pmol RNase Standard per well. Make up the wells to 54 µl with Molecular Biology Grade Water. Add 6 µl 10 X RNase Buffer into all Standard wells. *Volume for all Standard wells should be 60 µl.*
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well containing Sample(s), Positive Control, and Negative Control, prepare 10 µl Mix containing:

Sample Reaction Mix	
RNase Probe	1 µl
Molecular Biology Grade H_2O	9 µl

Mix and add 10 µl of the Sample Reaction Mix to each well containing the Samples, Positive Control and Negative Control. Mix well for 30 -60 seconds. Total volume for all wells (Samples, Control, Standard) = 60 µl.

4. **Measurement: For RNase contamination detection:** Measure initial (t = 0 hrs) fluorescence (Ex/Em = 495/520 nm) in endpoint mode. Place the cover of the plate (provided) on and incubate it between 1-3 hr. at RT avoiding light. Measure fluorescence at the desired incubation time (1-3 hr). **For activity quantification:** measure fluorescence (Ex/Em = 495/520 nm) in kinetic mode every 2 minutes for at least 30 - 60 minutes at 25 °C.
5. **Detection of RNase Contamination:** Make sure that there is no significant increase in negative control by avoiding cross-contamination in the Sample wells. Limit of detection of RNase is 0.4 pg/well: RNAse contamination can be defined as:

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

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.

Confirmation of results: Presence of RNase inhibitors or pH can affect the detection of contaminants and cause false negatives. Add 10 µl of the 10,000-fold dilution of the RNase positive control into suspected false negative wells. Incubate in the dark for 10 minutes at room temperature and measure the fluorescence (Ex/Em = 495/520 nm). The absence of RNAse inhibitors in Samples can be confirmed if:

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

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

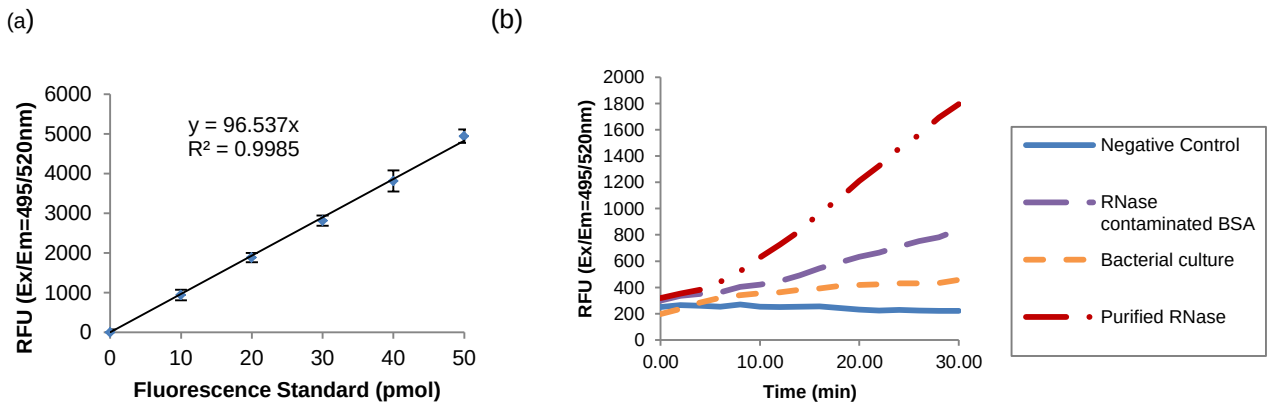
6. **Quantification of RNase Activities:** Plot the RNA Probe Standard Curve with pmol of RNA on the x-axis and RFU on the y-axis. Apply a linear fit to the RNase Standard values and determine the Standard Curve equation. *Samples/Positive Control:* Plot RFU on the y-axis vs. time (in minutes) on the x-axis and determine the slope (RFU/min) of the linear portion of the reaction curve. Apply slope (RFU/min) to the Standard Curve to obtain S (pmol/min). Estimate RNAse kinetic signal by subtracting Negative Control readings (NC) from Samples (S): **R=S-NC**.

Sample RNAse Activity = (R/V) x D (pmol/min/µl ≡ µU/µl)

Sample Specific Activity = R/(VxP) x D (pmol/min/µg ≡ µU/µg)

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

Unit Definition: One unit of RNAse activity is the amount of enzyme that cleaved 1.0 µmol of RNase Probe per min. at 25 °C.



Figures: (a) Fluorescence Standard Curve; (b) Sample kinetic curve obtained from Negative Control (Blank), bacterial culture, RNase contaminated BSA and purified RNase. Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS

K539 DNA Quantification Assay
K1351 EasyRNA™ Bacterial RNA Kit
K1373 EasyRNA™ Blood RNA Mini Kit
K1337 EasyRNA™ Cell/Tissue RNA Mini Kit
K1374 EasyRNA™ Plant RNA Mini Kit
K1418 Yeast RNA Mini Kit
K1419 EasyRNA™ Fungal RNA Kit

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