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Product Information

RNaseReveal™ Activity Assay Kit

Catalog Number: 31086

Kit Contents

Component	Size
31086A: RNaseReveal™ Substrate	1 x 1 mL
31086B: 10X RNaseReveal™ Assay Buffer	1 x 1 mL

Unit Size: 100 assays

Storage and Handling

Store at -20°C. Protect RNaseReveal™ Substrate from light. Kit components are stable for at least 6 months from date of receipt when stored as recommended.

RNaseReveal™ Substrate is an RNA-based substrate and precautions should be taken to avoid RNase contamination.

Product Description

The RNaseReveal™ Activity Assay Kit is a fluorescent assay for the detection of RNase activity in liquid samples. The kit utilizes RNaseReveal™ Substrate, which is an RNA probe tagged with a green fluorophore and a quencher, so that the intact probe is non-fluorescent. In the presence of RNase, the probe is cleaved and the fluorophore is detached from the quencher, releasing a green fluorescent signal. The buffer and substrate have been formulated for maximum sensitivity, making it a useful tool for quality control workflows or for detecting RNase contamination in solutions to be used with RNA work. The lower limit of detection of RNase in the assay is ~0.08 pg, using Biotium's RNase A (Cat. No. 99871-600uL) at ≥50 U/mg (Kunitz units). Full substrate cleavage is achieved with ~800 pg of the same RNase A.

The excitation and emission maximum of the substrate is 496/516 nm. The substrate can be detected by any fluorescence plate reader or fluorometer capable of detecting green fluorescence.

Experimental Protocols

Protocol outline

1. Incubate samples with substrate in microplate for 30 minutes at 37°C
- ↓
2. Read initial fluorescence ("RFUinitial")
- ↓
3. Add RNase to all wells and incubate 10 minutes at 37°C to maximally cleave the substrate
- ↓
4. Read maximum fluorescence ("RFUmax")
- ↓
5. Calculate the % maximum signal for the initial fluorescence values for all wells

Materials required but not provided

- RNase-free water (see Related Products)
- RNase A (see Related Products)
- Black 96-well microplate

Assay Protocol

When performing this assay for the first time, check to make sure your samples do not include any of the incompatible substances listed in Table 1 on page 2. It may also be helpful to include a well with 1 uL of RNase A added at the start of the assay in order to set an appropriate instrument gain (see notes to Step 5b).

1. Allow RNaseReveal™ Substrate and 10X RNaseReveal™ Buffer to thaw and reach room temperature.
2. Make a master mix of equal volumes of RNaseReveal™ Substrate and 10X RNaseReveal™ Buffer. Each well needs 20 uL of master mix (10 uL of Substrate + 10 uL of 10X Buffer).

Suggested experiment setup:

- a. Negative control (RNase-free water) - two wells
- b. Test sample(s) - two wells per test sample
- c. RNase-added control - one well (optional for establishing microplate gain setting, see step 5 for details)

Note: If using this control, take special care to avoid cross-contamination of samples with RNase. Prepare the negative control and sample wells before preparing the RNase-added control.

3. Pipette 20 uL of the master mix into the appropriate number of wells of a black 96-well plate.
4. Pipette 80 uL of sample into each well. Mix by pipetting up and down. Pipette 80 uL of RNase-free water into 2 wells as a negative control.

5. Insert the plate into a fluorescent microplate reader and set up the RNaseReveal™ assay as follows:

- a. Incubate for 30 minutes at 37°C (incubation can also be done externally in an incubator, protected from light).
- b. Read fluorescence at Ex/Em 490/520. This value will be “RFUinitial”.

Notes:

- i. Do not use autogain to maximize this signal. You will want to use the same setting later to read the “RFUmax” signal, which will be much brighter. Negative controls with RNase-free water are expected to have initial reads below 5% of the maximum signal.

- ii. The gain should be optimized for samples containing a saturating amount of RNase for detection of the fully cleaved substrate prior to running the experiment. When running the assay for the first time on a specific instrument, you may want to include a well with RNase added during the first incubation to set an appropriate gain to avoid saturation of the RFUmax reading.

6. Add 1 uL RNase A (≥100 ng/mL) to each well. Mix by pipetting up and down.
7. Return the plate to the microplate reader and resume the RNaseReveal™ program as follows:
 - a. Incubate for 5-10 minutes at 37°C (incubation can also be done externally in an incubator, protected from light).
 - b. Read fluorescence at Ex/Em 490/520. This value will be “RFUmax”.

8. Data analysis

- a. Calculate the % of maximum signal (%max) for each sample's initial read using the following formula:

$$\text{RFUinitial}/\text{RFUmax} * 100$$

- b. A sample without RNase contamination will have a %max similar to the water control. The %max for the water control and other negative samples should be <5%. Samples with RNase contamination will have roughly 5-100% maximum signal.

- c. The RFUmax value for all samples should be similar to the water control. If the value is significantly lower, it may indicate that the sample contains a component that is inhibitory to the RNaseReveal™ assay. You may wish to prepare a dilution of your sample in RNase-free water and test the diluted sample in the assay.

Table 1. Incompatible Substances

Incompatible substances	Notes
Darkly colored solutions	Darkly colored solutions may interfere with excitation or emission of the RNaseReveal™ Substrate
Solutions that inhibit RNase activity	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 RNaseReveal™ Substrate	Solutions with pH >9
	Caustic solutions (strong acids and bases, bleach)

Troubleshooting

Problem	Solutions
Very low RFUmax values for samples	The RFUmax value for all samples should be similar to the water control. If the value is significantly lower, it may indicate that the sample contains a component that is inhibitory to the RNaseReveal™ assay. You may wish to prepare a dilution of your sample in RNase-free water and test the diluted sample in the assay.
High %max values for water or negative controls	A sample without RNase contamination will have a %max similar to the water control. The %max for the water control and other negative samples should be <5%. If the water control has a %max >5%, try a new sample of water in case the previous sample was contaminated with RNase.

Related Products

Cat. No.	Product
99871-600uL	RNase A
41024-4L	Water, Ultrapure Molecular Biology Grade
22028	RNase-X™ Decontamination Solution
31073	AccuBlue® Broad Range RNA Quantitation Kit
41044	EMBER™ Ultra RNA Gel Kit
41032	EMBER500™ RNA Prestain Loading Dye
28001	ExoBrite™ EV Total RNA Isolation Kit
CD506	CELLDATA RNAsort™ 2.0 FFPE RNA Extraction Kit
CD510-96	CELLDATA RNAsort™ 2.0 MagBead FFPE RNA Extraction Kit
CD504	CELLDATA RNAsort™ Fresh Cell and Tissue RNA Isolation Kit
CD508	CELLDATA DNAsort™/RNAsort™ 2.0 Combination Kit

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