# 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:

      RFUinitial/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 RNAstorm™ 2.0 FFPE RNA Extraction Kit |
| CD510-96 | CELLDATA RNAstorm™ 2.0 MagBead FFPE RNA Extraction Kit |
| CD504 | CELLDATA RNAstorm™ Fresh Cell and Tissue RNA Isolation Kit |
| CD508 | CELLDATA DNAstorm™/RNAstorm™ 2.0 Combination Kit |

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