AttoTector Rapid RNase Activity Assay (RRAA)
Catalog Number: EZ2024-01

Kit Contents:

Component	Amount	Storage Condition
Assay Buffer	12 mL	-15°C to -25°C
Dual Labeled Oligo Substrate	200 µL	-15°C to -25°C
Positive Control	200 µL	-15°C to -25°C
96 well plate	1	Room Temperature

RRAA Test Method:

RNase activity in a convenient and sensitive fluorometric assay that delivers results in real time.

- Great for Quality Testing for RNase contamination of materials and supplies

RAA uses a novel RNA substrate tagged with a fluorescent reporter molecule (fluor) on one end and a quencher on the other.

In the absence of RNases, the physical proximity of the quencher dampens fluorescence from the fluor to extremely low levels. When RNases are present, however, the RNA substrate is cleaved, and the fluor and quencher are spatially separated in solution.

This causes the fluor to emit a bright green signal when excited by light of the appropriate wavelength. Fluorescence can be readily detected with a fluorometer. Since the fluorescence of the RAA Substrate increases over time when RNase activity is present, results monitored with a fluorometer can be evaluated kinetically.

Required Materials not provided:

All materials used should be RNase-Free. Micro-pipettes with disposable plastic tips (25-1000 µL), Multi-channel pipette (50-250 µL) or stepper pipette (50-250 µL), or electronic repeating pipette with disposable plastic tips, Timer, fluorescent microtiter plate reader (excitation 485nm and emission 525nm +/-5)

Assay Protocol:

Method control: It is best to run a set of negative and positive controls with each sample set run to ensure comparable readings from the day, time and user. Depending on the inhibitor being detected, a spike solution that can be used to generate the positive control can be made and added into control wells.

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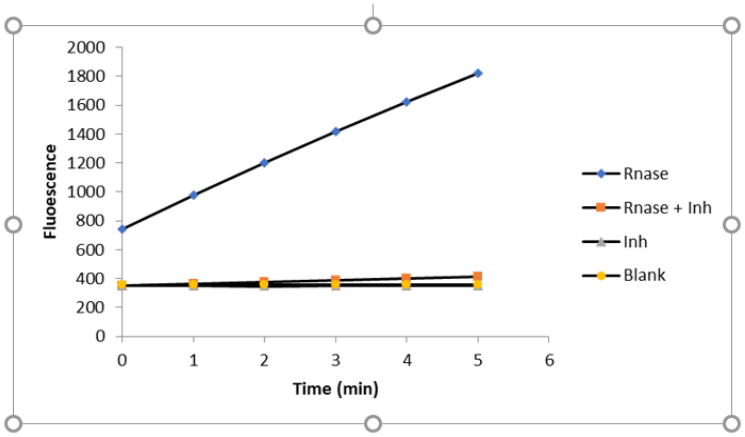
Protocol (volume for one well):

1. Dilute 2 µL oligo into 98 µL of Assay Buffer.
2. Add 100 µL of diluted oligo per well.
3. 20 µL sample or positive control per well.
4. Measure the change in fluorescence over a 10 min time interval using a fluorescent plate reader (excitation 485nm and emission 525nm).

The sequence of the RAA Substrate has been carefully optimized to detect several RNases, including RNase A, RNase T1, RNase I, micrococcal nuclease, S1 nuclease, mung bean nuclease, and Benzonase.



RRAA Substrates allow for rapid, sensitive detection of RNases and DNases. These in-house formulated reagents are fluorescence-quenched oligonucleotide probes that emit a fluorescent signal only after nuclease degradation. The assays can be read visually for qualitative assessment of contamination or by fluorometry for a quantitative measurement.

Qualitative measurements are useful for testing lab reagents, equipment, and supplies for nuclease contamination. The assays can be used quantitatively to study enzyme kinetics. The RRAA Substrate employs a FAM™ reporter (Em 520 nm), while the DNaseAlert Substrate employs a HEX™ reporter (Em 555 nm).