Rapid RNase Activity Assay (RRAA)
Catalog Number: NA2022

Kit Contents:

Component	Amount	Storage Condition
Assay Buffer	12mL	-20°C
Dual labeled oligo substrate	200 µL	-20°C
Positive Control	200 µL	-20°C
96 well plate	1	RT

Description:

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).

Rapid RNase Activity 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 2ul oligo into 98ul of assay buffer.
2. Add 100ul of diluted oligo per well.
3. 20ul 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.

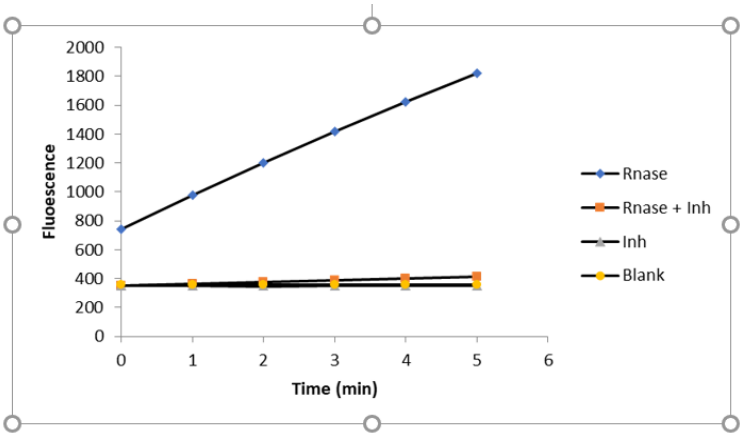

Figure 1. Analysis of RNase A activity over a 5-minute time interval using the RRAA test kit. Relative fluorescence increased in the presence of RNase A and was inhibited in the presence of RNase Inhibitor.