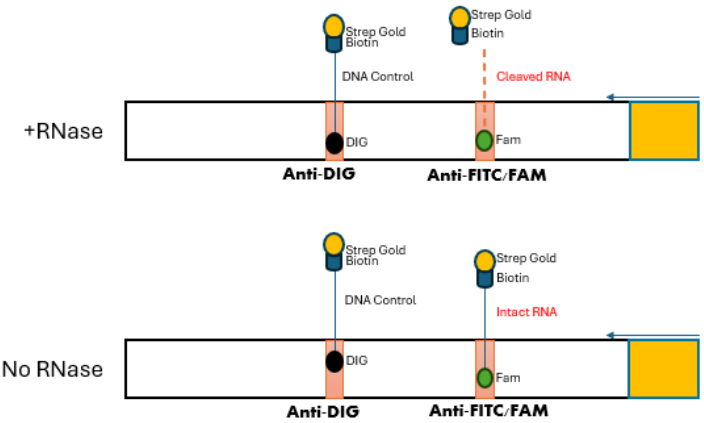
RNase Alarm Assay
Catalog Number: AU2042

Kit Contents:

Component	Amount	Storage Condition
RNase Alarm Reaction Buffer	2.5ml	RT
2X RNase Alarm Running Buffer	2.5ml	RT
RNase Free water	1.5mL	RT
96 well plate	1	RT
Lateral Flow Strips	50	RT
Bio-RNA-FITC	250ul	-20°C
Bio-DNA-Dig	250ul	-20°C

Description:



This patent pending test is designed for the sensitive and accurate analysis of RNase activity in liquid samples or solid surfaces. RNase Alarm uses a novel RNA substrate that attaches to the streptavidin colloidal reporter molecule (gold) using a 5' end biotin. The RNA also contains a 3' FITC/FAM molecule that enables it to be captured by the anti-FITC/FAM antibody (test line). In the absence of RNases, the RNA oligo tethers gold to the test line giving a visual test line. When RNases are present, the RNA substrate is degraded, and the gold particles can no longer be tethered to the test line thus, signal is lost. Since the cleavage of the RNA Substrate increases over time when RNase activity is present, results can be evaluated kinetically. This patent pending assay has applications for quality control testing and analysis of unit activities of both RNase and RNase inhibitors.

Required Materials Not Provided:

All materials used should be RNase-free. There are RNase-free certified products commercially available, and in-house RNase-free solutions can be made with appropriate handling, high grade reagents and ultrapure water.

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Micro-pipettes with disposable plastic tips (25-1000µL), multi-channel pipette (50-250µL) or stepper pipette (50-250µL), or electronic repeating pipette with disposable plastic tips, timer, and lateral flow strip reader. RNase A or RNase Inhibitors may be desired as controls in the test.

Rapid RNase Activity Assay Protocol:

Method control: It is best practice to have 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 sample being analyzed, an RNase A spike solution can be used to generate positive control. In the presence of RNase, the test line should diminish or disappear. In addition, an RNase Inhibitor treatment can be applied to detect inhibition activity.

Protocol (volumes for a single well):

1. Calculate the number of reactions you will be performing and break off the number of wells of the 96 well plate corresponding to this number of wells needed to perform the test.
2. Remove all the remaining strips not being used from the 96 well plate plastic frame and place them into the foil pouch bag for later use.
3. Add 50ul of RNase Alarm Reaction Buffer into each well.
4. Next add 5ul of Bio-DNA-Dig into each well.
5. Next add 5ul of Bio-RNA-FITC into each well.
6. Next add 5ul of sample, negative control, or positive control into their designated well.
7. Mix each reaction well by pipetting up and down using a new RNase-Free pipette tip for each reaction.
8. Cover the plate/wells with plastic wrap and incubate for 15 minutes at 37°C.
   Note: Incubation time can be increased or decreased to achieve the desired level of sensitivity.
9. Following incubation, add 50ul of 2X RNase Alarm Running Buffer and mix well.
10. Add one lateral flow strip into each well.
11. Run each strip for 15 minutes.
12. Review results by taking photos and/or analyzing them in a lateral flow strip reader such as the DETEKT RDS-2500 lateral flow reader.

The sequence of the RNA 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.

Precautions: To prevent RNase cross-contamination, use barrier tips and avoid splashing. Use RNase Free solutions and reagents if diluting samples. Ensure Bio-RNA-FITC is opened in a clean environment and kept away from contaminating ribonucleases.