RNaseFLOW Assay
Catalog Number: AU2042

Kit Contents:

Component	Amount	Storage Condition
RNaseFLOW Reaction Buffer	2.5ml	RT
2X RNaseFLOW 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
Bio-DNA-Dig	250ul	-20

Description:

This kit is designed for the sensitive and accurate analysis of RNAse activity in liquid samples or solid surfaces. RNaseFLOW 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 molecule that enables it to be captured by the anti-FITC 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. 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 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 sample being analyzed, an RNase A spike solution can be used to generate the positive control can be made and added into control wells. In the presence of RNase, the test line should diminish or disappear. In addition, a RNase Inhibitor can be applied to detect inhibition activity.

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Protocol (volume for one well):

1. Add 50ul of RNaseFLOW Reaction Buffer in a well of a 96 well plate.
2. Add 5ul of DNA into a well of the supplied 96 well plate.
3. Add 5ul of RNA into each well of the supplied 96 well plate.
4. 5ul sample, negative, or positive control into each well of the provided 96-well plate.
5. Incubate for 5 minutes. (Incubation time can be increased or decreased to achieve the desired level of sensitivity).
6. Add 50ul of 2X RNaseFLOW Running Buffer and mix well.
7. Add one lateral flow strip into each well.
8. Run for 15 minutes.
9. Review results by taking photo and/or analyzing in a lateral flow strip 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.