# RNase H Lateral Flow Assay Kit
# Catalog Number: AU2045

## Kit Contents:

| Component | Amount | Storage Condition |
|---|---|---|
| RNase H Reaction Buffer | 4ml | RT |
| 2X RNase H Running Buffer | 4ml | RT |
| RNase H Lateral Flow Strips | 50 | RT |
| RNase H Substrate | 250ul | -20 |
| Control Line Oligonucleotide | 250ul | -20 |
| RNase Free water | 1.5mL | RT |
| 96 well plate | 1 | RT |

## Description:

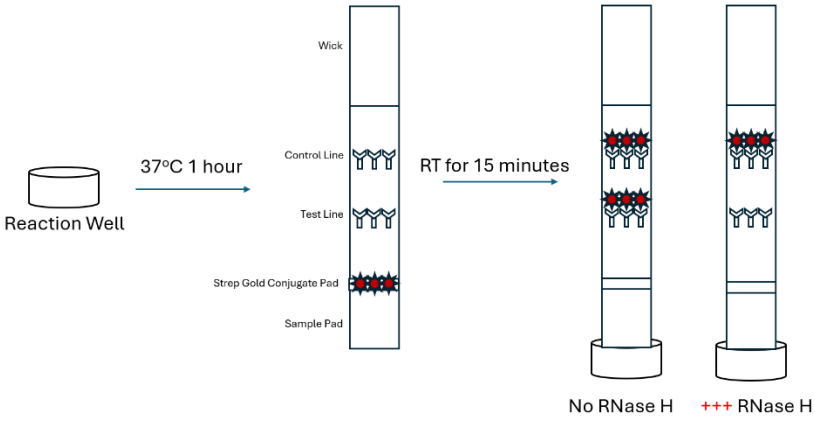


**Figure 1**. At the heart of the RNase H Lateral Flow Test is streptavidin conjugated colloidal gold that binds to a biotin labeled control oligonucleotide ssDNA (Biotin and Dig) and or a test line RNase H substrate RNA/DNA hybrid that contains a 5'-Biotin and 3'-FAM. These oligonucleotides are incubated in a reaction well containing optimized RNase H reaction buffer at 37°C for 1 hour. After the incubation, a lateral flow strip is dipped into the well and incubated (run) for 15 minutes at room temperature. In the presence of RNase H, the test line is decreased. In the absence of RNase H, both the test and control lines are present.

This kit is designed for the sensitive and accurate analysis of RNAse H activity in liquid samples. RNase H uses a novel RNA/DNA substrate that attaches to the streptavidin colloidal reporter molecule (gold) using a 5' end biotin. The RNA/DNA 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 (**Figure 1**). 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, a user-supplied RNase H spike solution can be used to generate the positive control can be made and added into control wells. In the presence of RNase H, the test line should diminish or disappear. In addition, User-supplied substrates can be applied to analyze substrate specificity.


| Units RNase H | Test | Control | Ratio |
|---|---|---|---|
| 10 units Rnase H | 1245186 | 156783 | 0.13 |
| 5 Units Rnase H | 1239324 | 117909 | 0.1 |
| 2.5 Units Rnase H | 1190578 | 105187 | 0.09 |
| 1.25 Units Rnase H | 1196661 | 154984 | 0.13 |
| 0.625 Units Rnase H | 1258886 | 337021 | 0.27 |
| 0.312 Units Rnase H | 1216396 | 710610 | 0.58 |
| 0.156 Units Rnase H | 1169827 | 934139 | 0.8 |
| 0.078 Units Rnase H | 988595 | 1173518 | 1.19 |
| 0.039 Units Rnase H | 935781 | 1426721 | 1.52 |
| No Rnase H | 852171 | 1274038 | 1.5 |

**Figure 2**. RNase H Lateral Flow Test was performed by mixing 75ul of RNase H reaction buffer with oligonucleotide substrates DNA and RNA/DNA, the indicated amount of RNase H in wells of a 96 well plate and incubated at 37°C for 1 hour. Following the 1-hour incubation, 75ul of 2X RNase H lateral flow running buffer was added to the well and mixed then strips were added into the wells and run for 15 minutes. Following 15 minutes, the samples were analyzed using a lateral flow strip reader. Data show the relative values of the test and control lines along with the ratio generated using the Detekt RDS 2500 Lateral Flow Assay Reader.

## Protocol (volume for one well):

1. Add 75ul of RNase H Reaction Buffer in a well of a 96 well plate.
2. Add 5ul of RNase H substrate into a well of the supplied 96 well plate.
3. Add 5ul of Control Line Oligonucleotide into each well of the supplied 96 well plate.
4. 1-5ul 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 75ul of 2X RNase 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.

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.