A First of its Kind Assay to Detect Ribonuclease H Activity and Substrate Preferences Using Lateral Flow Technology

Introduction:

As they might say on Sesame Street, this article is sponsored by the letter 'H'. We're not talking about the 'Preparation', but rather the Ribonuclease. Ribonuclease H (RNase H) is a class of enzymes that specifically catalyze the hydrolysis of the RNA-strand of an RNA-DNA hybrid. In addition to being identified in human cells, retroviral reverse transcriptases' naturally possess RNase H activity in addition to their well-described polymerase function. These enzymes transcribe the single stranded RNA viral genome into double stranded DNA, which then can be handled by the host cell like one of its own genes. The past decade has brought an escalating growth of important new commercial applications for RNase H enzymes and inhibitors of RNase H activity, including wide applications as targets for anti-viral therapeutics and reverse transcription applications.

Attogene has created a novel lateral flow test for the sensitive detection of RNase H (catalog number AU2045) with five very important advantages (https://www.attogene.com/shop/rnaseh-flow-activity-assay/). Our RNase H activity assay kit is (1) convenient (does not require expensive equipment), (2) saves time (can be performed in 15 minutes as compared to hours), (3) is sensitive (detects as few as 0.07 units of RNase H), (4) enables visual readouts which can be quantified using lateral flow strip readers, and (5) enables a high level of user flexibility in evaluating different substrates, enzymes, inhibitors/activators, and reaction conditions of interest. Altogether, these five advantages of our novel product will create a highly effective tool to further support continued innovation in this field. Our Attogene RNase H test has the ability to replace classical RNase H activity assays. For example, would you rather use radioactivity to assess the number of units of RNase H in a sample (the classic Hillenbrand and Staudenbauer assay), or a much simpler, quicker, and safer lateral flow assay? In addition, our Attogene RNase H assay can readily be used with combinations of enzyme concentrations and substrates of interest to optimize your R&D efforts.

Results:

Attogene has developed a first of its kind lateral flow assay to detect ribonuclease H activity. This assay uses streptavidin-conjugated colloidal gold (also sold in liquid form separately from Attogene – catalog number AU2017) that is embedded into the conjugate pad of the lateral flow strip. Part of this conjugate pad overlaps with the sample pad and nitrocellulose membrane to enable efficient flow between these aspects of the lateral flow assay. Sprayed onto the nitrocellulose are control and test line antibodies (e.g. anti Anti-Dig and anti-FAM) using our in-house lateral flow manufacturing equipment. A wick that overlaps with the upper section of the nitrocellulose is added to enable the efficient movement of the reaction components past the test/control lines at a defined pace. The kit also includes an optimized RNase H reaction buffer, optimized 2X assay running buffer, control oligonucleotide (5'-biotin-ssDNA-3'Dig), and a test line RNA/DNA hybrid oligonucleotide substrate (5'-biotin-RNA/DNA-3'FAM). To set up the reaction, the RNA/DNA hybrid and control DNA are simply incubated in the RNase H reaction buffer in the presence of a sample or control at 37°C for 1 hour. Following the incubation, 2x lateral flow assay running buffer is mixed into the reaction, a lateral flow strip is added into the well and incubated/run for 15 minutes. At the end of the 15 minutes, the test is analyzed by eye or by using a lateral flow reader. If ribonuclease H activity is present, the RNA/DNA duplex substrate is degraded, and colloidal gold can't be tethered between the gold through biotin/streptavidin and the test line by association with the anti-FAM. In turn, a reduction in test line signal is observed in the presence of ribonuclease H (**Figure 1**). The

decrease in test line is quantitative and directly relative to the amount of ribonuclease H activity in the sample. Importantly for some applications, this test format also allows for user-flexibility in which a user-supplied substrate can be added in place of the kit supplied substrate to evaluate RNA/DNA substrate specificity.

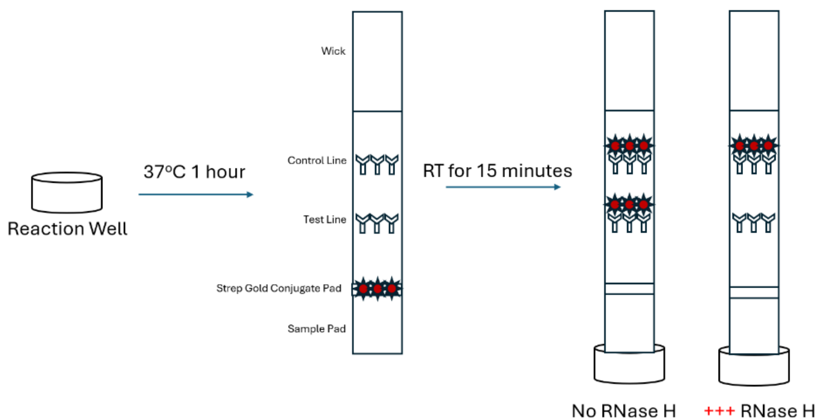
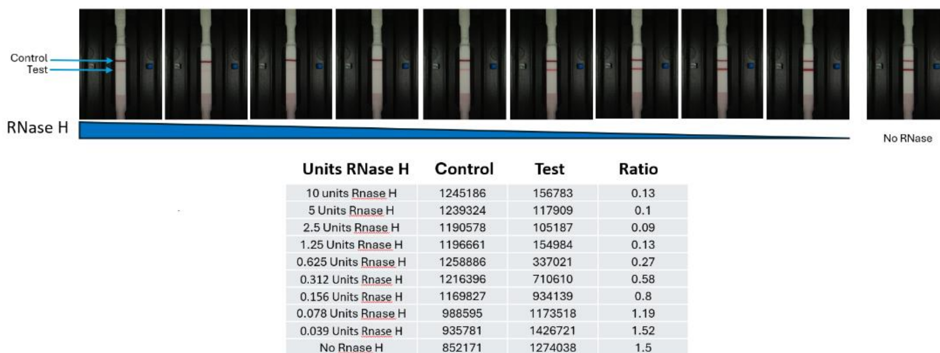


Figure 1. At the heart of the RNase H Lateral Flow Test is streptavidin conjugated colloidal gold that binds to a biotin labeled control oligonucleotide ssDNA (Biotin and Dig) and or a test line RNase H substrate RNA/DNA hybrid that contains a 5'-Biotin and 3'-FAM. These oligonucleotides are incubated 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.

In **Figure 2** we show data that demonstrates the sensitivity of the lateral flow RNase H activity assay using the Attogene kit supplied substrate and with Ambion RNase H (Thermo Fisher catalog number AM2292). To determine the limit of detection of our RNase H lateral flow assay, we added increasing concentrations of ribonuclease H into the reaction wells containing our RNase H reaction buffer, control/substrate oligonucleotides, mixed well, and incubated for 1 hour at 37°C. We then added our optimized 2X running buffer, added the lateral flow strip and incubated it for 15 minutes. Strips were then read using the Detekt RDS 2500 lateral flow assay reader and numerical readouts, and the pictures were included as a reference (**Figure 2**). As you can see, our lateral flow RNase H activity assay can easily detect as low as 0.07 units of RNase H.



Units RNase H	Control	Test	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.

Discussion:

We envision that a major use of the Attogene RNase H test will be as a tool for experimental testing of RNase H enzymes to determine aspects like enzyme activity, substrate preference/specificity, the effect of base modifications on RNA/DNA substrates, RNase H inactivation, and RNase H drug activity testing. The Attogene RNase H bypasses the need for radioactivity, expensive fluorescent readers and importantly for some users, the need to run gels!