



Universal Lateral Flow Assay Kit

For Laboratory Use with User Supplied Streptavidin

Conjugated Reporter-Particles

Catalog Number: AU2064-01, 02, and 03

For Research Use Only. Not for use in Diagnostic Procedures.

I. Introduction

Attogene's universal lateral flow assay kit is a ready-to-use, universal test strip (dipstick), which is based on the lateral flow technology that uses reporter particles such as colloidal gold/silver, Europium Beads, Phycoerythrin, Quantum Dots, latex beads etc., conjugated to streptavidin to conveniently capture nucleic acid molecules. The dipstick is designed to conveniently develop qualitative or quantitative rapid test systems for detection of nucleic acids with secondary capture antibody.

Detection of nucleic acid (DNA or RNA) using this system requires the use of a biotin and fluorescein isothiocyanate or 6-carboxyfluorescein (FITC/FAM)-labelled nucleic acid for the AU2064-01-02-03-kit versions, nucleic acids containing biotin and digoxigenin (dig) for the AU2064-02-03-kit versions, and nucleic acids containing biotin and 2, 4-Dinitrophenol (DNP) for the AU2064-03-kit version only (**Figure 1**). In creating nucleic acids using amplification, the primers should be designed so that the final product contains both moieties on the final product. Test line: anti-FITC/FAM, Control Line: Biotin. For multiplex detection (02 kit version) of nucleic Acid (DNA or RNA) requires the use of one nucleic acid containing biotin, FITC or FAM and another containing biotin and dig: Test Line #1: anti FITC/FAM, Line #2: anti-Dig, Control Line Biotin. For multiplex detection (03 kit version) of nucleic Acid (DNA or RNA) requires the use of one nucleic acid containing biotin, FITC or FAM, biotin and dig, or biotin and DNP: Test Line #1 anti-DNP, Test Line #2: anti-FITC/FAM, Test Line #3: anti-Dig, Control Line: Biotin.

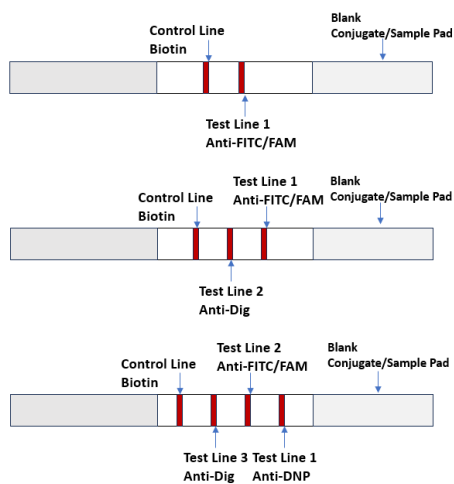


Figure 1. Demonstrates the single-plex and multiplex versions of the kits.



Figure 2. This data demonstrates the importance of the relative amounts of molecules of a synthetic duel labeled oligo (containing biotin and FAM) with the amount of gold particles to finding optimal line intensities. Biotin line captures streptavidin reporter-particles when streptavidin is not occupied with the duel labeled oligo. The more duel labeled oligo added, the binding to the anti-FITC/FAM reaches maximal intensity at 1pmol and then diminishes due to over saturation with the duel labeled oligo above 4 pmoles, free biotinylated oligo overtakes the system to bind both the streptavidin gold and anti-FITC/FAM while also occupying every gold particle in the system leading to a loss in intensity of both the biotin line and the anti-FITC/FAM line also called the “hook effect” (data not shown).

you add more biotin labeled nucleic acid product into the reaction the less control line is visualized. Therefore, a comparison of the sample should be made with a strip containing only sample running buffer in each experiment. The appropriate concentration of labeled nucleic acid to use with strips is dependent upon the purity and sequence of the nucleic acid and a standard curve can be used to determine the relative ratio. A positive control duel labeled (biotin-FITC/FAM, biotin-dig, or biotin-DNP) nucleic acid of your sequence that has a well characterized size, purity and concentration can be used for comparison.

Features & Benefits

- Can be used for development of a lateral flow assay for detection of a variety of different molecules such as amplified DNA products from PCR, LAMP and RPA reactions.
- No need to stripe capture antibodies
- No expensive equipment required
- Cost-effective way to screen for further downstream lateral flow assay development.

The sample containing the nucleic acids to be detected is simply mixed into the specially designed nuclease free assay running buffer in a well of the supplied 96-well plate, mixed with the user provided reporter-particle conjugate, and the dipstick is then added. Generally, the reaction is complete in 10-15 minutes. It is important to note that the relative stoichiometry between the nucleic acid added and the reporter-particle is important in assay optimization (**Figure 2**).

This test can be run for a ratio analysis as

2. Kit Contents

Component Name	Volume	Storage
4.5mm Dipsticks	50 each	RT
Control Nucleic Acid containing biotin and FAM (AU2064-01, AU2064-02, and AU2064-03 kit)	20 µL	Refrigerate
Control Nucleic Acid containing biotin and DIG (AU2064-02 and AU2064-03 kit only)	20 µL	Refrigerate
Control Nucleic Acid containing biotin and DNP (AU2064-03 kit only)	20 µL	Refrigerate
96 well plate	1 each	RT
Manual	1 each	RT

3. Storage and Stability

- The kit should be stored at 2°C - 4°C until ready to use.
- The test strips must remain in the desiccated vial until use.

4. Required Materials Not Supplied

- Timer - For timing use
- Centrifuge - For preparation of clear specimens
- Pipettor and pipette tips – to transfer samples and controls

5. Precautions

- Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
- The test strips are packaged in a desiccated vial. Do not remove strips unless they are to be used subsequently for testing.
- Avoid cross-contamination of samples by using a new tube and disposable pipette tip for each sample.
- Use only Lateral Flow Kit reagents from one kit lot, as they have been adjusted in combination.

- It is good laboratory practice to use positive and negative controls to ensure proper test performance.
- Due to the hook effect, if no signal is detected in the test line, a serial dilution may be necessary to bring the nucleic acid into the appropriate concentration ratio/stoichiometry with the gold and the test line capture reagents to see the test line.

7. Procedure

Perform the following:

1. Add 100-150 μ L of Running Buffer into a well of a 96 well plate. **Note:** Attogene provides a variety of different running buffers, including RNase Free variants, suitable for use with Colloidal Gold, Quantum Dots, Phycoerythrin, and Europium Beads in this assay. These are available for purchase with their respective particles, or you may contact us for pricing on the individual buffers themselves. AU2035 offers samples of five different RNase free running buffers for screening.
2. Add your streptavidin reporter-particle conjugate to each well. **Note:** Required amounts of reporter-particles will vary depending on the type and concentration used. For a 10 OD solution of Colloidal gold, 2-10 μ L is typically a good starting point. For fluorescent particles at a 1mg/mL or 1 μ M concentration 0.5-2 μ L is a good starting point.
3. Always run a positive and negative control well with the sample:
 - A. (SAMPLE) mix a designated amount (a volume 1 μ L-5 μ L are good starting points) of product into the sample running buffer. When running an LFA for the first time, we recommend trying large dilutions of sample/antigen to determine the dynamic range of the assay and keeping the volume below 15 μ L if possible. If a greater volume of sample is required when running the assay, a more concentrated buffer may need to be used.
 - B. (POSITIVE CONTROL) mix 5 μ L of the control oligonucleotide
 - C. (NEGATIVE CONTROL) leave this well blank (don't add any sample or control)
4. Mix each well completely by pipetting up and down several times.
5. Add one dip stick into each well (arrows facing up).
6. Incubate for 10-15 minutes

7. Visually analyze the strip by eye, photography or read in a lateral flow reader.

NOTE: If a higher analytical sensitivity is required, it could be helpful to increase the volume and/or concentration of the nucleic acid added into the well. Volume and concentration of analyte-specific solutions, and incubation time are always part of the individual test development.

NOTE: Control oligonucleotide line should yield a test and control line signal on the strip within 15 minutes.

8. Interpretation of Results

This test is a lateral flow assay containing test lines that are dependent on the concentration of biotin-FITC/FAM (for AU2064-01, AU2064-02, and AU2064-03 kit), biotin-DIG (for AU2064-02 and AU2064-03 kit), and biotin-DNP (for AU2064-03 kit only) labeled nucleic acids in the sample.

What to expect at the test lines:

The higher the concentration of nucleic acid in the sample the higher the intensity of the test line compared to the strip lacking nucleic acid (negative control strip).

What to expect at the control line:

The intensity of the control will decrease as the test line increases.

Determination made using strips which have dried for more or less than the required time may be inaccurate, as line intensities may vary with drying time.

9. Additional Analysis

If necessary, positive samples can be confirmed by analyzing using nucleic acid analysis techniques such as agarose or acrylamide gels. A lateral flow reader may also be employed to generate numerical readings.

Who we are:

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

Contact Us

3913 Todd Lane, Suite 310
Austin, TX 78744

Phone: 512- 333-1330

Email: sales@attogene.com

Web: www.attogene.com

AU2064-XX_VI_03_20_2025