Europium Based Fluorescent Universal Lateral Flow Assay Kit

Catalog Number: AU2049

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

1. Introduction

Attogene's fluorescent universal lateral flow assay kit is a ready-to-use, universal test strip (dipstick), which is based on the lateral flow technology that uses fluorescent particles (broad range UV light excitation range of 300nm to 400nm, 610nm emission) containing streptavidin that can be detected with commercially available black lights (such as those sold on Amazon) 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. Fluorescent based lateral flow assays have been described to be 2-100x more sensitive than gold based lateral flow assays.

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 - AU2049-01 and AU2049-02-kit versions or nucleic acids containing biotin and digoxigenin (DIG) for the 02-kit version (**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/FAM and another containing biotin and DIG: Test Line #1: anti FITC/FAM, Line #2: anti-DIG, Line #3 Biotin.

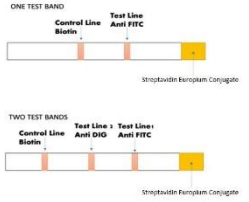

Figure 1. General diagram of the single plex or multi plex universal LFA dipstick.

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 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 streptavidin conjugated fluorescent particle is important in assay optimization. This test can be run for a ratio analysis as the more streptavidin labeled nucleic acid product that is added 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 or biotin-DIG) nucleic acid of your sequence that has a well characterized size, purity and concentration can be used for comparison.

2. Features and 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.

3. Kit Contents

Component Name	Volume	Storage
4.5mm Dipsticks	50 each	RT
Nucleic Acid Lateral Flow Running Buffer	12 mL	RT
Control Nucleic Acid containing biotin and FAM (01 and 02 kit)	20 µL	Refrigerate or Freeze
Control Nucleic Acid containing biotin and DIG (02 kit only)	20 µL	Refrigerate or Freeze
96 well plate	1 each	RT
Manual	1 each	RT

4. Storage and Stability

- The kit should be stored at 2°C - 30°C until ready to use.
- The test must remain in the sealed pouch until use.

5. Required Materials Not Supplied

- Timer - For timing use
- Centrifuge - For preparation of clear specimens
- Pipettor and pipette tips – to transfer samples and controls
- Molecule of interest containing Biotin and either FITC/FAM (01 version) or DIG (02 version).
- Tubes or microtiter plates to run the strips

6. 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 foil pouch with desiccant.
- 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 effects, 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 Europium and the test line capture reagents to see the test line.

7. Procedure

Perform the following:

1. Add 200µL of Nucleic Acid Lateral Flow Running Buffer into a well of a 96 well plate.
2. Always run a positive and negative control well with sample
   A. (SAMPLE) mix a designated amount (a volume 1µL-5µL are good starting points) of product into the sample running buffer. When running a 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 15µL if possible.
   B. (POSITIVE CONTROL) mix 5µL of the control oligonucleotide
   C. (NEGATIVE CONTROL) leave this well blank (don't add any sample or control)
3. Mix each well completely by pipetting up and down several times.
4. Add one dipstick into each well (arrows facing up).
5. Incubate for 15-30 minutes
6. Visually analyze the strip by eye by shining with a black light or read in a fluorescent lateral flow reader. The Test Line Signal will most likely diminish rapidly once the strips are taken out of the well. Due to this, immediate analysis is highly recommended to

achieve optimal read outs. Specialized readers are needed to obtain the 2-100x increased levels of sensitivity described in the literature.

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 01 and 02 kit) and/or biotin-DIG (for 02 kit) 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 a nucleic acid analysis technique 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

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