



**Fluorescent Europium Based Universal Human
IgG/IgM Lateral Flow Assay Kit
For Laboratory Use
Catalog Number: AU2052**

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

I. Introduction

Attogene's Europium Based Fluorescent Human IgG/IgM universal lateral flow assay kit is a ready-to-use, universal test strip, which is based on the lateral flow technology that uses Europium Dyed Nanoparticles containing streptavidin to conveniently capture biotinylated antigens. The device is designed to easily develop qualitative or quantitative rapid test systems for detection of anti-human IgG and IgM antibody that reacts to any antigen that can be biotinylated (i.e. viral antigen, autoimmune antigen) and is easily customizable providing every laboratory with the possibility to perform the assay feasibly.

Antibody tests are a common method of choice to determine if a person has been exposed to a pathogen or not. They are also incredibly valuable in the detection of autoantibodies that can be found in human autoimmune disorders. In this test, a biotinylated antigen (User supplied) is mixed with a biotinylated rabbit IgG (binds to goat anti rabbit control line) and sample (human sera or plasma) is simply mixed into with the specially designed assay running buffer in a well of the supplied 96-well plate, mixed and is then added to the sample port of the cassette. Generally, the reaction is complete in 30 minutes. It is very important to note that the relative stoichiometry between the biotinylated antigen, biotinylated rabbit IgG added, and the streptavidin Europium is critical for assay optimization. The appropriate concentration of biotinylated antigen to use with strips is dependent upon the purity and sequence and a standard curve can be used to determine the relative ratio (generally between 1ng-100ng per test). A positive control line (biotin-rabbit IgG) antibody will bind to the goat anti rabbit (GAR) line on the test to ensure the assay is running appropriately.



Features & Benefits

- Can be used for development of a lateral flow assay for detection of anti-human antibody targeting the antigen of choice.

- No need to stripe capture antibodies.
- Cost-effective way to screen for further downstream lateral flow assay development.

2. Kit Contents

Component Name	Volume	Storage
lateral flow dipsticks	50	RT
Assay Running Buffer	15 mL	RT
Biotin Rabbit IgG (control line antigen 1ng/ul)	250 µL	Refrigerate
Biotin Human IgG (IgG Control)	15 µL	Refrigerate
Biotin Human IgM (IgM Control)	15 µL	Refrigerate
Manual	1 each	RT
96 Well Plate	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 container until use.

5. Required Materials Not Supplied

- Biotinylated antigen(s)
- Samples (contrived sample, human sera, or plasma)
- Centrifuge - For preparation of clear specimens
- Pipettor and pipette tips — to transfer samples and controls.

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 vial that contains desiccant. Avoid prolonged periods of having vial open to avoid desiccant becoming saturated.
- 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 biotinylated antigen into the appropriate concentration ratio/stoichiometry with biotinylated rabbit IgG to see a test line and a control line at the desired intensity.

8. Procedure

Prepare serially diluted biotinylated antigen in phosphate buffered saline (i.e., 1 µg/ml to 1 ng/mL). Prepare the clinical (e.g., human serum or plasma) or contrived (e.g., humanized IgG) sample.

Perform the following:

1. Add 300 µL of Lateral Flow Running Buffer into a well of a 96 well plate.
2. Always run a positive and negative control well with sample:
 - A. (TEST LINE REAGENTS) mix a designated amount (a volume 1 µL-5 µL are good starting points) of biotinylated antigen into the assay 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 15 µL if possible. As controls for the human IgG or human IgM capture lines, use 5 µL of control biotin labeled human IgG or human IgM.
 - B. (CONTROL LINE REAGENT) mix 5 µL of biotinylated rabbit IgG (5 ng total)
 - C. (SAMPLE) Add between 1-10 µL clinical (e.g., human serum or plasma) or contrived (e.g., humanized IgG) sample.
3. Mix each well completely by pipetting up and down several times.
4. Place test strip into well and run vertically.
5. Incubate for a minimum of 30 minutes. If the test line is weak, or non-specific reactivity is still visible, it may be necessary to increase the incubation period.
6. Visually analyze the strip by eye in conjunction with an ultraviolet flashlight or photograph in a fluorescent strip reader.

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

9. Interpretation of Results

This test is a lateral flow assay containing test lines that are dependent on the concentration of antibody in the sample. Adjustment of sample may be required to understand the dynamic range of the test.

What to expect at the test lines:

The higher the concentration of human anti IgG or IgM reactive against the biotinylated antigen in the sample the higher the intensity of the test line compared to the strip run in a mixture lacking sample (negative control strip).

What to expect at the control line:

The intensity of the control will not decrease as the test lines increase.

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.

10. Additional Analysis

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

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