Universal IgG Lateral Flow Assay – Instructions for Use

25 lateral flow assay cassettes, Research Use Only

INTENDED USE

The Universal IgG Lateral Flow Assay is a highly sensitive Research Use Only device intended to detect human IgG antibodies specific for any given antigen. The user selects the antigen of choice, which must be biotinylated, and adds it to the sample. This technique allows the user to rapidly customize the test to any antigen of interest.

BENEFITS

Antibody tests are a method of choice to determine if a person has been exposed to a pathogen or not. However, developing a lateral flow assay requires expertise and access to dedicated manufacturing equipment which is not necessarily available in every laboratory. The Universal IgG Lateral Flow Assay provides every laboratory with the possibility to run their own antibody test. All parameters, such as the choice of nanoparticles, materials, and surfactants, have been optimized to provide maximum sensitivity when detecting antibodies in whole blood, plasma, or serum.

The platform is rapidly customizable to any antigen if it can be biotinylated. As a result, the user can validate a new antigen with a minimal financial investment and can rapidly create a business case for his/her discovery.

YOUR CHOICE

The Universal IgG Lateral Flow Assay comes with no strings attached. If you want us to dry your antigen within the test strip, so that you may have your own rapid test, ready to be commercialized under your name, we will gladly do so.

PRINCIPLE

The strip (Figure 1) contains:

1. Dark colored nanoparticles (gold nanoshells), conjugated to a mouse monoclonal anti-human-IgG antibody and subsequently dried within a water-soluble matrix.
2. A blood filter that retrains red blood cells present in the sample and prevents them from migrating to the assay.
3. A nitrocellulose strip containing a test line of polystreptavidin and a control line of anti-mouse antibody.
4. An absorbent pad.

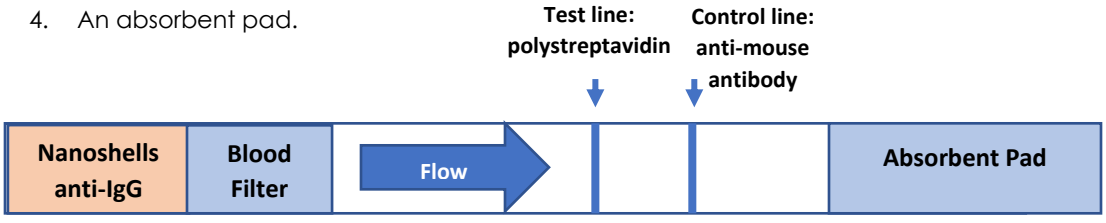

Figure 1. Universal IgG strip design.

When a sample contains IgG antibodies specific for a given antigen, and when that antigen is also present and is tagged with biotin, then a complex "human IgG + biotinylated antigen" is formed. That complex is then added on top of the blood filter.

Next, the chase buffer is added on top of the nanoshells-anti-IgG conjugate. This triggers several events. The "human IgG + biotinylated antigen" complex is carried along the nitrocellulose strip and gets trapped at the polystreptavidin test line (Figure 2). All other antibodies are washed off the strip, and only the human IgG specific for the antigen under scrutiny remain bound to the test line. In addition, the nanoparticles are released, and as they migrate towards the test line, they bind to the human IgG present there. The accumulation of nanoparticles at the test line results in a visual readout. The absorbent pad wicks off the excess moisture.

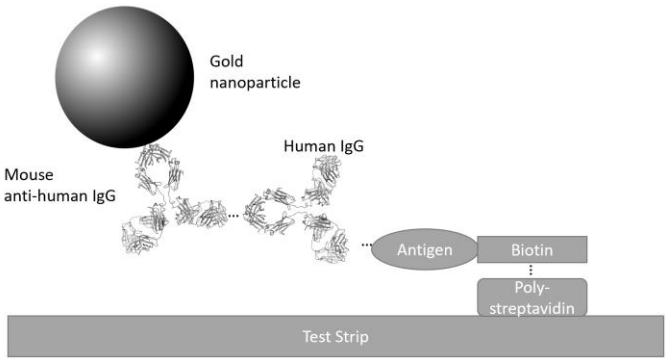

Figure 2. Principle of detection. At the test line, polystreptavidin captures the biotinylated antigen, which itself binds the human IgG to be detected. The latter binds to the anti-human IgG antibody coating the surface of the gold nanoparticles. The accumulation of colored nanoparticles at the test line leads to a visual signal.

MATERIALS AND REAGENTS

- 25 cassettes to detect antibody-antigen binding.
- Dropper bottle containing 4 ml of Chase Buffer.

MATERIALS NOT PROVIDED

- Biotinylated antigen(s).
- Phosphate Buffered Saline.
- Negative Human Serum.
- Single-channel adjustable volume pipette.
- Pipette tips.
- Laboratory timer.
- Test and control samples.

PRECAUTIONS

- The devices should remain in the closed pouch until use.
- Do not freeze any component of the test.
- Dispose device according to local regulations.
- For Research Use Only (RUO) – not an In Vitro Diagnostic (IVD).

STORAGE & STABILITY

The Universal IgG Lateral Flow Assay is projected to be stable for at least 1 year at room temperature, but this has not been formally demonstrated yet. We therefore recommend storing the devices at 4–8 °C. After storage at 4 °C bring the pouch to ambient temperature before opening. The cassettes should remain in the pouch with silica packs until use. Do not use after the expiration date.

DIRECTIONS FOR RUNNING THE ASSAY

STEP 1: Prepare biotinylated antigen and samples

1. Prepare serial dilutions of biotinylated antigen in phosphate buffered saline, covering the 0.5–50 µg/ml range (typically 0.5, 1, 5, 10, and 50 µg/ml).
2. If the sample is a clinical sample (blood, serum, plasma), do not dilute it.
3. If the sample is a contrived sample made by diluting a recombinant positive control (*e.g.* humanized IgG) in negative human serum, start at a 20 µg/ml concentration.
4. Remove the cassettes from the aluminum pouch and reseal the pouch.

STEP 2: Select the optimal amount of biotinylated antigen as follows:

1. In an Eppendorf tube, mix 5 µl of negative human serum with 5 µl of biotinylated antigen. We recommend sweeping the 0.5–50 µg/ml antigen concentration range.
2. Add the mixture of negative human serum + biotinylated antigen (10 µl) to the round sample port (Figure 3, port 1).
3. Let the mixture absorb into the pad (10–30 seconds).
4. Add 2 drops (or 54 µl) of Chase Buffer to the rectangular buffer port (Figure 3, port 2).

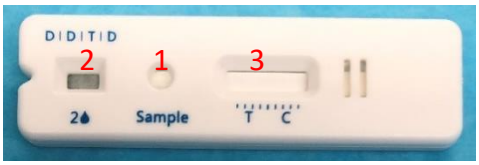

Figure 3. Universal IgG test cassette design. (1) sample port, (2) chase buffer port, (3) test results window and areas where the test and control lines are to be expected, T and C respectively.

5. Allow the Rapid Test to develop for 30 minutes.
6. Ideally, the test line should remain invisible at all concentrations of biotinylated antigen.
7. If some concentrations of biotinylated antigen induce nonspecific binding while others do not, select the maximum concentration that does not induce nonspecific binding (= no visible test line).
8. If all concentrations of biotinylated antigen yield nonspecific binding, then our platform is unlikely to be adaptable to your antigen.

STEP 3: Test your sample:

1. Mix 5 µL of sample with 5 µL of biotinylated antigen.
2. Add the mixture to the round sample port (Figure 2, port 1).
3. Let the mixture absorb into the pad (10–30 seconds).
4. Add 2 drops (or 54 µL) of Chase Buffer to the rectangular buffer port (Figure 2, port 2).
5. Interpret results after 30 minutes (see below).
6. The test line will slightly increase in intensity (about 25 %) over the next 15 minutes. We typically do not recommend reading the results after 1 hour.
7. Verify that the same test samples, when run without biotinylated antigen, do not give a positive test line.

VISUAL INTERPRETATION OF RESULTS

A control line is included in each test strip as a procedural control. The presence of this control line indicates a correct procedural technique. The absence of the control line after 20 minutes indicates an invalid result.

POSITIVE: There will be a distinct test line (T), and a control line (C). The color intensity of the test line will vary based on the concentration of the specific IgG present in the sample.

NEGATIVE: There will be only one distinct line at the control region C in the test result window.

INVALID: There will be no control line.

Figure 4. Interpretation of the universal IgG test results. The biotinylated antigen is SARS-CoV2 RBD protein (Acros Biosystems, cat. No. SPD-C82E9) diluted 1:80 (2.5 µg/ml) in saline. Positive: The test sample is anti-SARS-CoV2 Spike protein (Sino Biological cat. No. 40150-D001) diluted 1:1000 (1 µg/mL) in negative human serum (Cone Bio cat. No. 4090). Negative: The test sample is undiluted negative human serum (Cone Bio cat. No. 4090).

QUALITY CONTROL

Our tests are verified with the reagents indicated in figure 3; these reagents are commercially available.

CUSTOMER SERVICE

For any questions regarding the universal IgG test, please contact us at quality@ddtd.org.