Universal Lateral Flow Assay Kit
PRODUCT DATA SHEET

Universal Lateral Flow Assay Kit

Cat No: AKIT-U

General Description and Assay Principle

Universal Lateral Flow Assay Kit is a convenient ready-to-use kit for the quick and costeffective development of a lateral flow dipstick assay for detection of proteins, antibodies, and amplified DNA products. The approach involves PCR amplification of the species-specific target DNA sequence and the detection of the amplicon using a lateral flow strip. The approach has been previously used for the species identification of fish, horse, pork, beef, sheep and pork samples.

The kit requires the user to design and optimize a specific detection strategy for the analyte to be detected, e.g., for a DNA amplicon or antigen. See limitations and requirements section and figure 1 and 3, respectively.

The dipstick strips supplied in the kit have a biotin capturing molecule immobilized on the membrane of the strip (test line) and anti-FITC conjugated gold nanoparticles dried down on the conjugate pad on the dipstick. When a sample containing analyte complexed or labeled with biotin and FITC labels is applied to the test strip it will solubilize the dried gold conjugate which will first bind to the FITC label. Through capillary forces the sample will then migrate up the test strip and the biotin label will be captured at the test line. The resulting red line will indicate the presence of a complexed FITC/biotin analyte in the sample, figure 1 and 3.

Kit Components

- 25 Universal Lateral Flow Dipsticks
- 2 x 1.5 mL Lateral Flow Running Buffer
- 25 mL Sample Dilution Buffer

1378 US-206 Ste 6-126, Skillman, NJ USA
Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

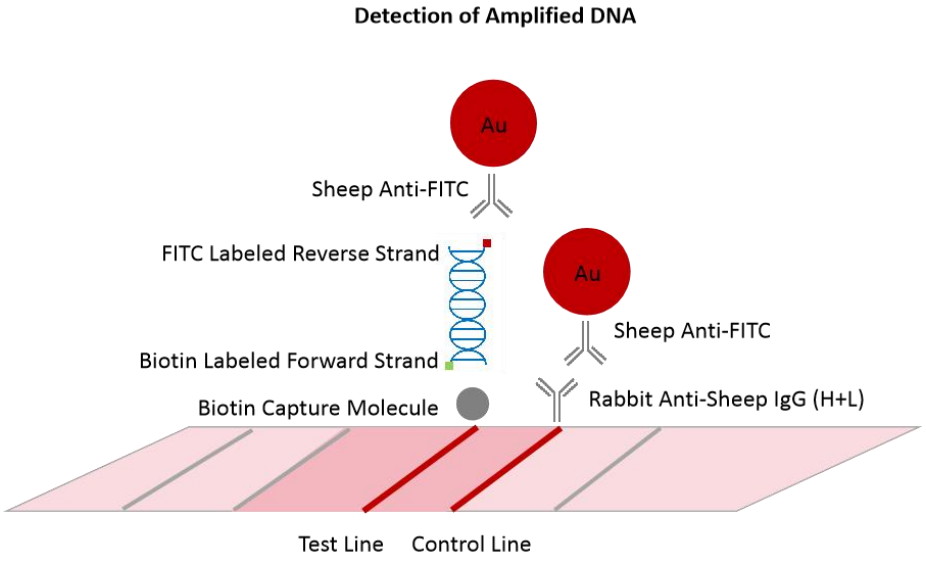

Figure 1. Schematic of the Universal Lateral Flow Assay Dipstick and its use for detection of a PCR amplified product.

Limitations and Requirements

Detection of proteins and other antigens: Requires the use of one biotinylated, and one FITC-labelled antibody against the protein target to be detected (not supplied with kit, analyte specific). The two antibodies must bind to different epitopes on the target (consult your specific antibody provider to ensure you are using a "matched pair").

Detection of amplified DNA product: Requires the use of a biotin and a FITC-labelled primer during amplification (not supplied with kit, target specific).

Note: FAM-labelled primers can be used in place of FITC-labelled primers if needed.

Storage and Stability

The dipsticks supplied in the Universal Lateral Flow Dipstick Kit should be stored between 2-24°C and the supplied Lateral Flow Assay Buffer and Sample Dilution Buffer should be stored at 2-8°C. If stored properly, this kit is stable for at least 3 months.

1378 US-206 Ste 6-126, Skillman, NJ USA
Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

Example Protocol for Detection of Amplified DNA

Protocol

1. Dilute 0.1-1 µL* of PCR product to 50 µL using the supplied sample diluent.

*Note: The amount PCR product used in the assay might need to be optimized and should be experimentally evaluated. The kit can successfully detect a FITC and biotin-labeled amplicon with a concentration in the range of 0.05 nM ~ 0.15 µM.

2. Transfer 100 µL of Lateral Flow Running Buffer into a well of a microtiter plate.

3. Add the diluted PCR product from step 1 into the well with Lateral Flow Running Buffer. Mix well via pipette.

4. Place a lateral flow dipstick into the well. The strip should be placed into the solution with the arrows pointing down.

5. Incubate for 10-20 min.

6. Remove lateral flow dipstick from the well. Some solution might remain in the well and is normal.

7. Immediately record the results using a lateral flow reader, or visually (qualitatively) using a lateral flow score card.

The appearance of two clearly visible red lines on the strip (control line (upper) and test line (lower)) indicates the successful detection of PCR product, see figure 2. A failed PCR reaction will only result in the formation of one red line at the control line.

See the troubleshooting section for assistance if needed.

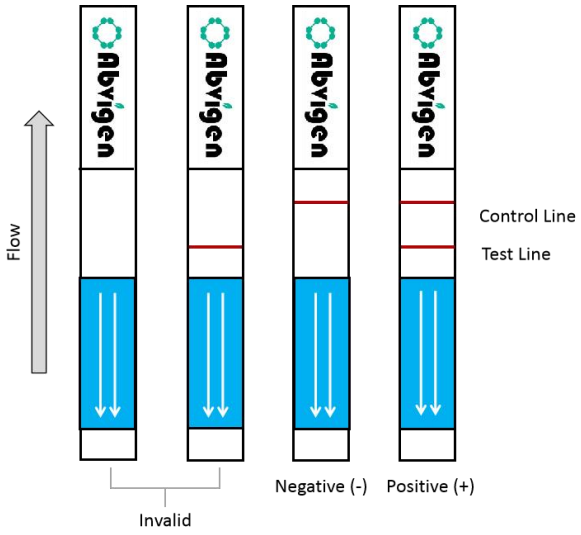

Figure 2. Possible lateral flow assay test outcomes. A valid test is either negative (a, control line visible) or positive (b, control and test lines visible). Invalid tests show only the test line (c) or no red lines (d) after assay completion.

1378 US-206 Ste 6-126, Skillman, NJ USA
Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

Example Protocol for Detection of Antigens (Sandwich Assay)

Protocol

1. Dilute sample with analyte to a final volume of 50 µL with the supplied sample diluent.

As a starting point during development the recommended final concentration of analyte in the sample should be in the range of 0.2 nM – 0.15 µM but should be optimized for each analyte and antibody pair used for detection.

2. Transfer 100 µL of Lateral Flow Running Buffer into a well of a microtiter plate.

3. Add *0.075 µg of FITC labeled detection antibody (5 µL @ 15 µg/mL) and *0.075 µg of biotinylated capture antibody (5 µL @ 15 µg/mL) to the well with Lateral Flow Running Buffer.

*Note: The quantity of detection antibodies might need to be optimized for each analyte and antibody pair used for detection.

4. Add the diluted sample with analyte from step 1 into the well with Lateral Flow Running Buffer and antibodies. Mix well via pipette.

5. Place a lateral flow dipstick into the well. The strip should be placed into the solution with the arrows pointing down.

6. Incubate for 10-20 min.

7. Remove lateral flow dipstick from the well. Some solution might remain in the well and is normal.

8. Immediately record the results using a lateral flow reader, or visually (qualitatively) using a lateral flow score card.

The appearance of two clearly visible red lines on the strip (control line (upper) and test line (lower)) indicates successful detection of the antigen, see figure 2. When no antigen target is present the assay will only result in the formation of one red line at the control line.

See the troubleshooting section for assistance if needed.

1378 US-206 Ste 6-126, Skillman, NJ USA
Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

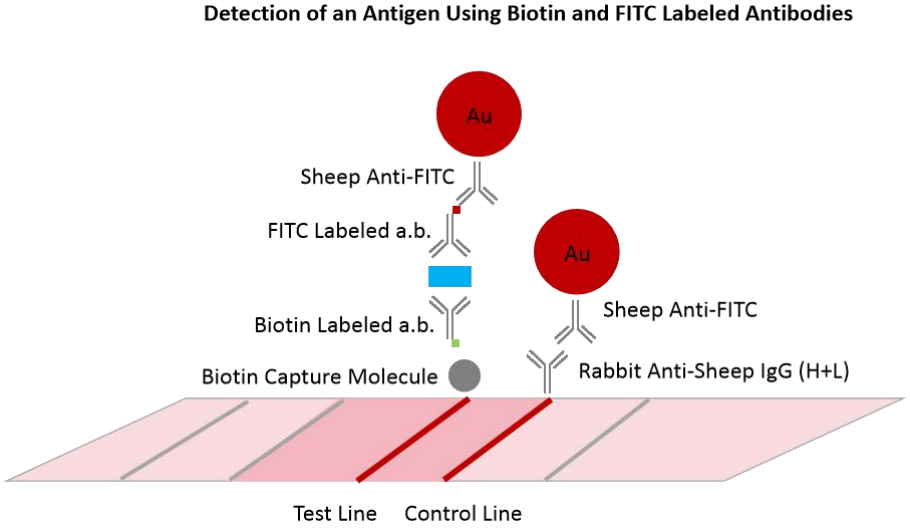

Figure 3. Schematic of the Universal Lateral Flow Assay Dipstick and its use for detection of an antigen in conjunction with one biotin-labelled antibody and one FITC-labelled antibody.

Troubleshooting

Problem Detected	**Possible Causes and Solutions**
No Signal Produced on the TestLine	**Detection of Amplified DNA** 1) There is too much or too little of the target present in the assay. We recommend beginning with a final amplicon concentration in the range of 0.05 nM ~ 0.15 µM. However, if there is too much amplicon present, the excess DNA may interfere with signal production within the assay (Hook Effect). If there is too little DNA, there may not be enough individual strands to sufficiently bind to the nanoparticle conjugate for detection. SOLUTION: Test final amplicon concentrations outside of the provided range. **Detection of Antigens** 1) The antibodies used are not a matched pair. Typically, when antibodies are geared towards use in a sandwich-style detection format, the supplier will list the

1378 US-206 Ste 6-126, Skillman, NJ USA
Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

	antibodies as being a “matched pair.” This means that it is confirmed that the antibodies will bind to separate domains on their intended target, rather than competing for the same binding spot on the same target. If the two antibodies are competing, then there will be no signal produced on the test line since the sandwich will not successfully form. SOLUTION: Confirm with your antibody provider that the antibodies used are a matched pair. 2) There is too much target present in the assay. We recommend starting with a target concentration in the range of 2 nM ~ 15 µM. However, if there is too much of the target protein present, the sandwich may not successfully form. SOLUTION: Reduce the target protein’s final concentration by factors of 10 (ie. 2 nM, 0.2 nM, 0.02 nM etc…) while holding the amount of antibodies constant. If a signal begins to appear, continue to reduce until the signal disappears (lower limit of detection). 3) The amount of antibodies used in the assay needs to be optimized. It may be that the amount of each labelled antibody used in the assay needs to be optimized. If there is too little antibody, then the antibodies may bind separate proteins, rather than the same one. If there is too much antibody, then the extra, unused biotinlabelled antibody may flow up the membrane and bind to the test line before the entire labelled sandwich can. This would result in the lack of a test line signal. SOLUTION: Hold the concentration of the protein constant while testing different amounts of antibody (ie. 0.025 µg,

1378 US-206 Ste 6-126, Skillman, NJ USA
Email: info@abvigenus.com
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161

	0.75 µg, 7.5 µg etc…). Note: both antibodies should be added in equal amounts to the well. Select a protein concentration well within the range that you desire for detection.
High Background Signals in Assay	1) The provided running buffer is incompatible with the analyte, antibodies, or ssDNA (due to the pH or salt level of the buffer). SOLUTION: Investigate and try out other running buffers that may have been used in similar assays. 2) Reagent additives are leading to non-specific binding during the assay. SOLUTION: Purify the antibody or analyte prior to use via a desalting column or centrifugal filter unit.

Disclaimer

While our **Universal Lateral Flow Assay Kit** is an excellent starting point for your assay development, validating your final assay is important. This kit includes all necessary components and a detailed protocol to guide you, but please note that it is not a guarantee of success. Variability in antibodies, antigens, oligos, and buffers can affect the outcome of your assay.

Ordering Information

Website: www.abvigen.com

Phone: +1 929-202-3014

Email: info@abvigenus.com