

PRODUCT DATA SHEET

Universal Lateral Flow Assay Kit

Catalog Number LF-019-10

Kit Components

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

General Description and Assay Principle

Cytodiagnosics' Universal Lateral Flow Assay Kit is a convenient ready-to-use kit for quick and cost-effective development of a lateral flow dipstick assay for detection of proteins, antibodies, and amplified DNA products.

The kit requires the user to design 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.

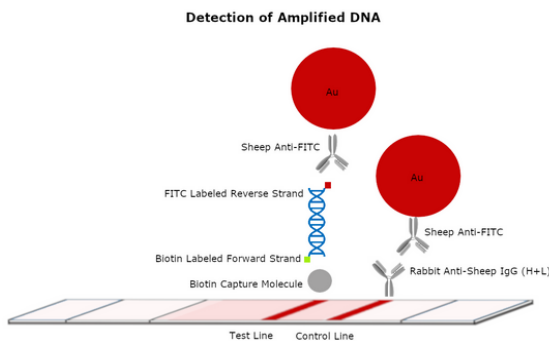


Figure 1. Schematic of the Universal Lateral Flow Assay Dipstick and its use for detection of a PCR amplified product.

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).

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

Storage and Stability

The dipsticks supplied in the Universal Lateral Flow Dipstick Kit should be stored between 2-30°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.

Example Protocol for Detection of Amplified DNA

Protocol

1. Dilute 0.1-1 ul* of PCR product to 50 ul 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 uM.

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.
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 minutes.
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 e.g. 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 PCR product, see figure 2. A failed PCR reaction will only result in the formation of one red line at the control line.

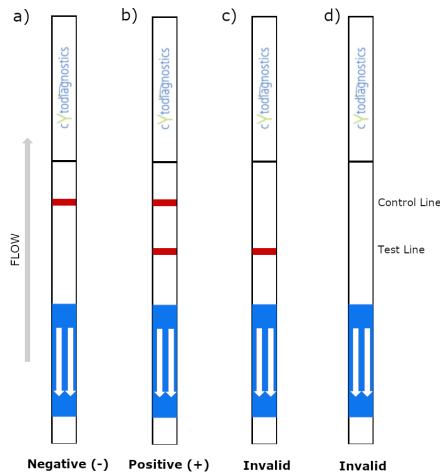


Figure 2. Possible lateral flow assay test outcomes. A valid test is either negative (a, control line visible) or positive (b, control and test lines visible). Invalid tests show only the test line (c) or no red lines (d) after assay completion.

Example Protocol for Detection of Antigens

Protocol

1. Dilute sample with analyte to a final volume of 50 μ l 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 μ M but should be optimized for each analyte and antibody pair used for detection.
2. Transfer 100 μ l of Lateral Flow Running Buffer into a well of a microtiter plate.
3. Add *0.075 μ g of FITC labeled detection antibody (5 μ l @ 15 ng/ml) and *0.075 μ g of biotinylated capture antibody (5 μ l @ 15 ng/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.
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 minutes.
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 e.g., 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.

Detection of an Antigen Using Biotin and FITC Labeled Antibodies

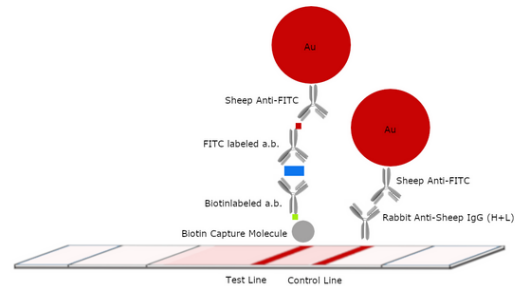


Figure 3. Schematic of the Universal Lateral Flow Assay Dipstick and its use for detection of an antigen in conjunction with one biotin-labelled antibody and one FITC-labelled antibody.