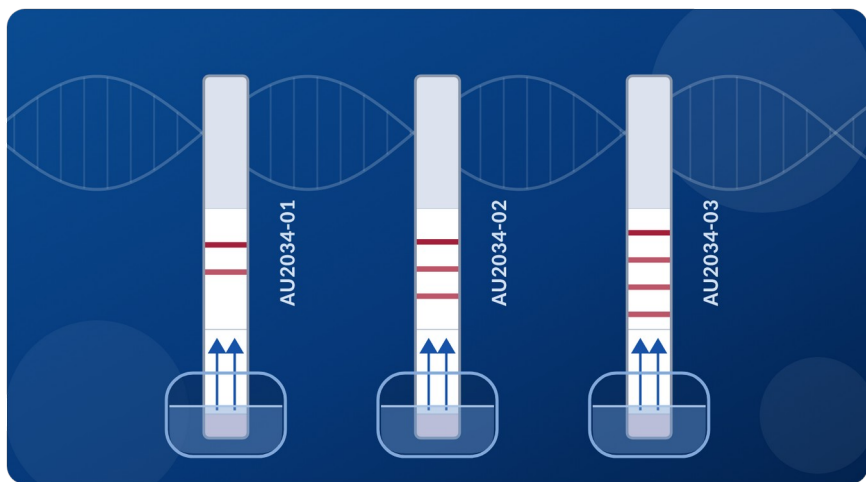




Universal Lateral Flow Assay Kit

For Laboratory Use

Catalog Number: **AU2034-01/02/03**

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



1. Introduction

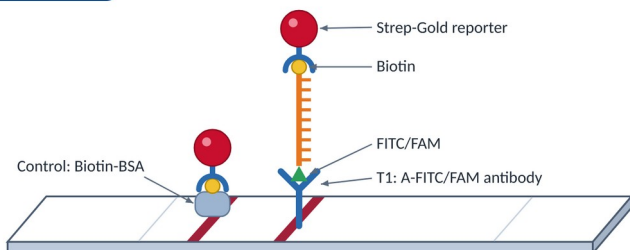
Attogene's universal lateral flow assay kit is a ready-to-use, universal test strip (dipstick), which is based on the lateral flow technology that uses gold particles containing streptavidin to conveniently capture biotin-labeled nucleic acid molecules. The dipstick is designed to conveniently develop qualitative or semi-quantitative rapid test systems for detection of nucleic acids with secondary capture antibody.

Detection of nucleic acid (DNA or RNA) using this system requires the use of a nucleic acid labeled with biotin and fluorescein isothiocyanate or 6-carboxyfluorescein (FITC/FAM) for the AU2034-01/02/03 kit versions, a nucleic acid labeled with biotin and digoxigenin (DIG) for the AU2034-02/03 kit versions, or a nucleic acid labeled with biotin and 2,4-dinitrophenol (DNP) for the AU2034-03 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.

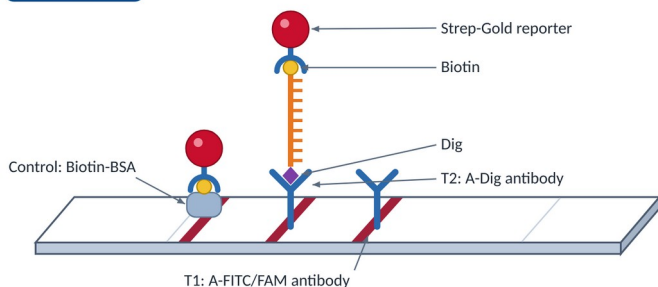
The sample containing the nucleic acids to be detected is simply mixed into the specially designed RNase-free assay running buffer in a well of the supplied 96-well plate, mixed and the dipstick is then added. Generally, the reaction is complete in 15 minutes. Unless your application has shown a different read time to be optimal, do not run the assay longer, as non-specific results may occur. It is important to note that the relative stoichiometry between the nucleic acid added and the streptavidin gold is important in assay optimization (Figure 2). This test can be run for a ratio analysis as the more dual-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 dual-labeled (biotin and FITC/FAM, biotin and DIG, or biotin and DNP) nucleic acid of your sequence that has a well characterized size, purity and concentration can be used for comparison.

AU2034-01

2-Line


AU2034-02

3-Line


AU2034-03

4-Line

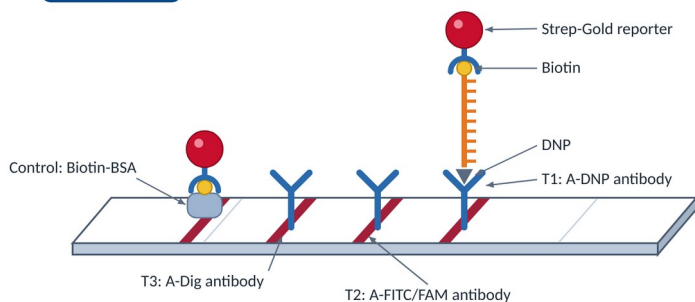
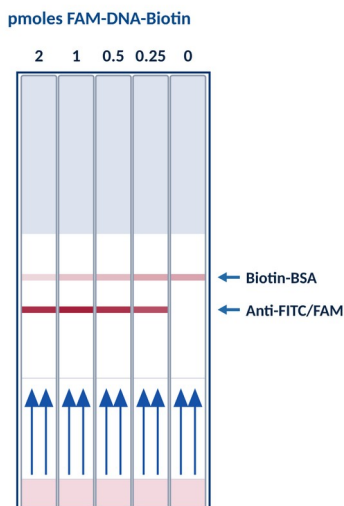


Figure 1. Capture schematics for the AU2034-01 (2-line), AU2034-02 (3-line) and AU2034-03 (4-line) dipsticks.



Concentration	Analyte Used	Control Line	Test Line	Ratio
2 pmoles	FAM-DNA-Biotin	43349	582050	13.43
1 pmoles	FAM-DNA-Biotin	108958	629684	5.78
0.5 pmoles	FAM-DNA-Biotin	132328	571066	4.32
0.25 pmoles	FAM-DNA-Biotin	198921	478063	2.40
0 pmoles	FAM-DNA-Biotin	204927	3699	0.02

Figure 2. Effect of the amount of a synthetic dual-labeled DNA oligonucleotide (5' 6-FAM, 3' biotin) on line intensities; strip images are redrawn from the reader values in the table. The biotin-BSA control line captures streptavidin gold that is not bound to labeled nucleic acid, so the control line weakens as the test line strengthens. In this example the test line is near its maximum from 0.5 to 2 pmol while the control line keeps falling, so the test-to-control ratio continues to rise. At very high amounts, free labeled molecules can saturate both the gold and the capture line and weaken both lines (the "hook effect"). How much excess a strip tolerates depends entirely on the specific product being run, so determine the working range for each new product.

Features & 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.

2. Kit Contents

Component Name	Volume	Storage
4.5 mm Dipsticks	50 each	RT
Nucleic Acid Lateral Flow Running Buffer	12 mL	RT
Control Nucleic Acid containing biotin and FAM (AU2034-01/02/03 kit)	20 µL	Refrigerate
Control Nucleic Acid containing biotin and DIG (AU2034-02/03 kit)	20 µL	Refrigerate
Control Nucleic Acid containing biotin and DNP (AU2034-03 kit)	20 µL	Refrigerate
96 well plate	1 each	RT
Manual	1 each	RT

Packaging: the kit ships in one pouch. Inside are the dipsticks in one desiccated canister, the 96-well plate in its own pouch, and the running buffer and control nucleic acids in a small ziplock bag.



3. Product Specifications

Key specifications for experienced users and for setting up a lateral flow reader.

Parameter	Specification
Format	Dipstick, 4.5 mm wide × 60 mm long; 50 strips per kit
Reporter	40 nm colloidal gold conjugated to streptavidin; lines develop red-purple
Test line capture	Anti-FITC/FAM (binds FITC/FAM), anti-digoxigenin (binds DIG) and anti-DNP (binds DNP), depending on the kit
Control line	Biotin-BSA; captures streptavidin gold that passes the test lines
Line spacing	4.5 mm, center to center, on all kits
Running buffer	150 µL per test; 12 mL supplied
Sample volume	1–5 µL recommended to start; keep below 15 µL
Time to result	Read at 15 minutes, unless your application shows that a different read time is optimal
Positive controls	Dual-labeled DNA oligonucleotides, 5 µL per test: Biotin and FITC/FAM: 0.02 pmol/µL (0.1 pmol per test); biotin and DIG: 0.003 pmol/µL (0.015 pmol per test) Biotin and DNP: 0.02 pmol/µL (0.1 pmol per test) Test and control lines appear within 15 minutes
Compatible samples	DNA or RNA carrying biotin plus FITC/FAM, DIG or DNP, including PCR, LAMP and RPA products
Storage	Dipsticks and running buffer at room temperature; control nucleic acids refrigerated (2–8 °C); dipsticks in their desiccated canister

Line positions (center, mm from the dip end of the strip)

Line	AU2034-01	AU2034-02	AU2034-03
Control (C): biotin-BSA	32.0	32.5	34.0
Test: anti-Dig	—	28.0 (T2)	29.5 (T3)
Test: anti-FITC/FAM	27.5 (T1)	23.5 (T1)	25.0 (T2)
Test: anti-DNP	—	—	20.5 (T1)

T-numbers match Figure 1.

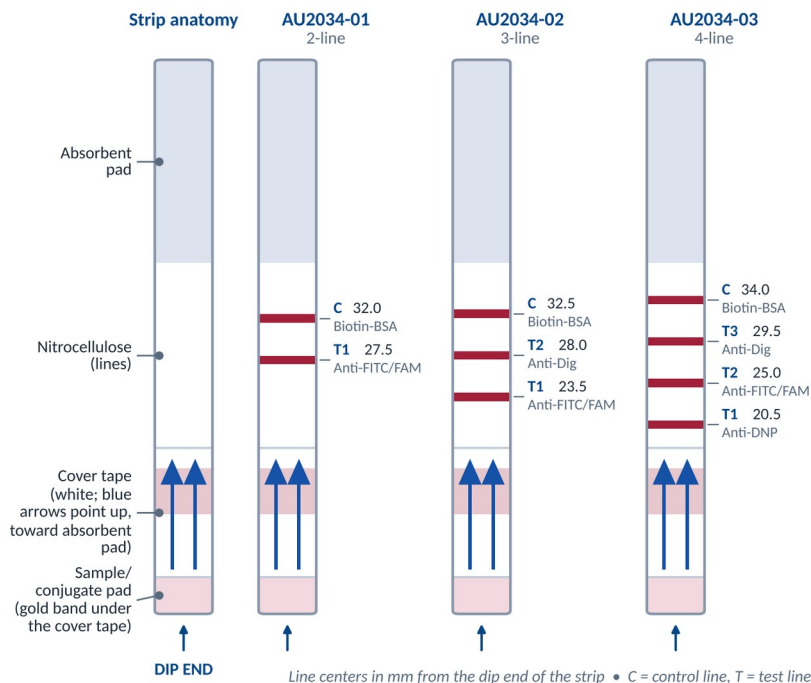


Figure 3. Strip layout for AU2034-01, -02 and -03 (lengths to scale; strip width exaggerated). Dip the sample/conjugate pad end into the well. The white cover tape carries blue printed arrows that point toward the absorbent pad, in the direction of flow.

4. Storage and Stability

- Store the dipsticks and the running buffer at room temperature.
- Store the control nucleic acids refrigerated (2–8 °C).
- Keep the dipsticks in the desiccated canister until use. Close the canister promptly after removing strips.

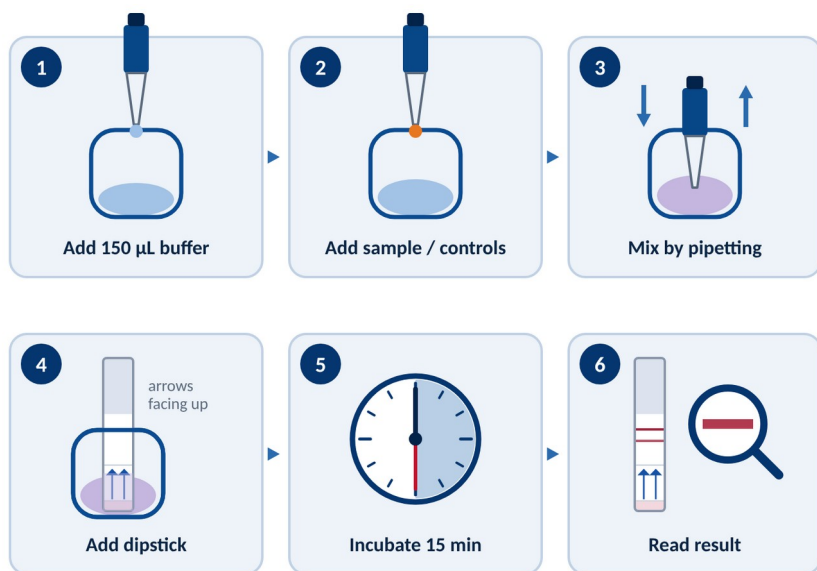
5. Required Materials Not Supplied

- **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 FITC/FAM (01/02/03 version), biotin and DIG (02/03 version), or biotin and DNP (03 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 dipsticks are packaged in a desiccated canister inside the kit pouch.
- 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 gold and the test line capture reagents to see the test line.

7. Procedure



Perform the following:

1. Add 150 µL 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 1 μL – 5 μL 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 15 μL if possible.
 - B. **(POSITIVE CONTROL)** mix 5 μL 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 minutes
 6. Visually analyze the strip by eye, photography or read in a lateral flow reader.

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 and FITC/FAM (for AU2034-01/02/03 kit), biotin and DIG (for AU2034-02/03 kit), or biotin and DNP (AU2034-03 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.

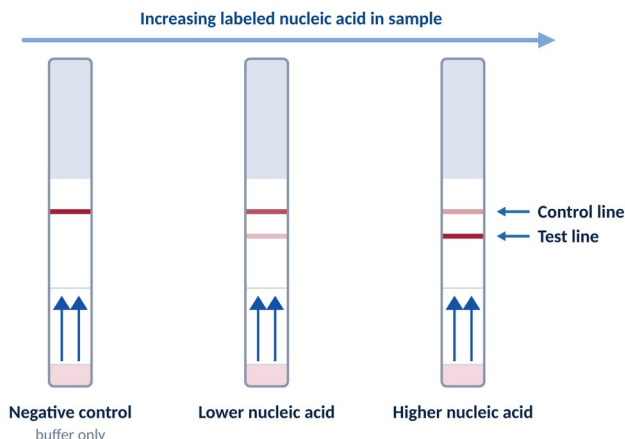


Figure 4. Illustration of expected line behavior (2-line format shown). Within the working range, test line intensity rises and control line intensity falls as more labeled nucleic acid is added; see Figure 2 for the hook effect at excess analyte.

Read the strip at 15 minutes, unless your application shows that a different read time is optimal.

9. Designing Labeled Nucleic Acids

Every molecule detected by this kit must carry two labels: biotin, which binds the streptavidin gold, and one capture label, which binds a test line. A molecule with only one of the two labels does not produce a test line.

Capture label	Test line	Kits	Positive control
FITC/FAM	Anti-FITC/FAM (T1 on -01 and -02; T2 on -03)	-01, -02, -03	biotin and FAM
Digoxigenin (DIG)	Anti-Dig (T2 on -02; T3 on -03)	-02, -03	biotin and DIG
DNP	Anti-DNP (T1 on -03)	-03	biotin and DNP

Primer and probe design

- **PCR:** label the 5' end of one primer with biotin and the 5' end of the opposite primer with the capture label, so every full-length amplicon carries both. Attogene's 874 bp control amplicon (NA2032) uses this design: biotin-labeled forward primer, FAM-labeled reverse primer.
- **LAMP and RPA:** apply the same rule. Place biotin and the capture label on primers (or a probe) that end up in the same double-stranded product.

- **Multiplexing (AU2034-02 and -03):** put biotin on one primer of every target and give each target its own capture label. Each target then develops at its own test line, and all targets share the gold and the control line.

Controls and stoichiometry

- **No-template control:** always include one. Primer-dimers or non-specific products that carry both labels produce a test line without target.
- **Free labeled primers:** unincorporated biotin-labeled primers occupy gold, and unincorporated capture-labeled primers occupy test-line binding sites. If amplification is strong but the test line is weak, dilute the product or lower the labeled primer concentration.
- **Shared gold:** all targets compete for the same gold, so a very abundant product can weaken the other lines and the control line (Figure 2). Dilute until every line is within its working range.
- **Reference amounts:** each 5 μ L positive control delivers 0.1 pmol (biotin and FAM), 0.015 pmol (biotin and DIG) or 0.1 pmol (biotin and DNP) of dual-labeled oligo. In Figure 2, the test line of the FAM-DNA-biotin oligonucleotide is near its maximum from 0.5 pmol.



10. Troubleshooting

Observation	Likely cause	What to do
No control line	Strip inserted upside down, not enough buffer in the well, or strip exposed to moisture	Insert the dip end down with the arrows pointing up; use 150 μ L of buffer; use a new strip from a closed desiccated canister
Test and control lines both faint at high sample input	Excess labeled nucleic acid (hook effect)	Run a serial dilution of the sample (Figure 2)
Test line in the negative control (buffer only)	Carry-over of labeled product into the well or buffer	Use a new tip for every well; keep amplified products away from buffer stocks and unused strips
Test line in the no-template amplification control	Labeled primer-dimers or non-specific amplification	Optimize or redesign the primers; check product size on a gel (Section 11)
Weak test line despite good amplification	Too little product, or free labeled primers competing	Increase the sample volume (up to 15 μ L) or dilute the product to reduce free primers
Smeared lines, high background or slow flow	Sample volume above 15 μ L, or a viscous or particulate sample	Keep the sample below 15 μ L; centrifuge to clear the sample before adding it
Lines change after the read time	Strip read later than the read time	Read at 15 minutes, or at the read time validated for your application

11. 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.



12. Ordering Information

Catalog no.	Description	Size
AU2034-01	Universal Lateral Flow Assay Kit, streptavidin gold, 2-line (anti-FITC/FAM; biotin-BSA control)	50 tests
AU2034-02	Universal Lateral Flow Assay Kit, streptavidin gold, 3-line (anti-Dig, anti-FITC/FAM; biotin-BSA control)	50 tests
AU2034-03	Universal Lateral Flow Assay Kit, streptavidin gold, 4-line (anti-Dig, anti-FITC/FAM, anti-DNP; biotin-BSA control)	50 tests
Add-on	Running buffer, 2X	10 mL
Add-on	Control nucleic acid: biotin and FAM, biotin and DIG, or biotin and DNP	200 µL each
Add-on	Cassetted strips (CO2022-02): the kit's strips supplied in lateral flow cassettes	50 cassettes

Add-ons are ordered with the kit. For bulk strips, custom line configurations or other formats, contact sales@attogene.com.



Contact Us

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Austin, TX 78744

Phone: 512-333-1330

Email: sales@attogene.com

Web: www.attogene.com



13. Quick Reference

Procedure at a glance

Step	Action
1	Add 150 μ L of running buffer to a well.
2	Add the sample (start with 1–5 μ L; keep below 15 μ L). Positive control: 5 μ L of control nucleic acid. Negative control: buffer only.
3	Mix by pipetting up and down several times.
4	Insert one dipstick per well, dip end down, arrows pointing up.
5	Incubate for 15 minutes.
6	Read by eye, by photograph or with a lateral flow reader.

Line map (top to bottom)

Kit	Lines
AU2034-01	C (biotin-BSA) · T1 anti-FITC/FAM
AU2034-02	C (biotin-BSA) · T2 anti-Dig · T1 anti-FITC/FAM
AU2034-03	C (biotin-BSA) · T3 anti-Dig · T2 anti-FITC/FAM · T1 anti-DNP

Reading the strip

- **Test line:** compare with the negative control strip; a stronger test line means more labeled nucleic acid at that capture line.
- **Control line:** weakens as the test line strengthens. This is expected (Figure 4).
- **No lines at all:** the strip did not run correctly. Repeat with a new strip (Section 10).

Storage

- Dipsticks and running buffer at room temperature; control nucleic acids refrigerated (2–8 °C).
- Keep dipsticks in the desiccated canister; close the canister promptly after removing strips.





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Take your nucleic acid lateral flow further

Reagents, controls and development tools from the team that builds the Universal Lateral Flow Assay Kit.



SCALE UP

Streptavidin Colloidal Gold
AU2017

40 nm, 10 OD streptavidin gold, the reporter in this kit, for your own lateral flow development or final product. Includes 50 mL RNase-free running buffer per mL.



SISTER KIT

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The same 2-, 3- and 4-line strip layout with anti-FITC/FAM gold and a streptavidin test line. 100 tests per kit.



CONTROLS

FAM/Biotin 23S Amplicon Control DNA
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874 bp end-labeled PCR product (biotin forward, FAM reverse primer): a full-length amplicon control for the anti-FITC/FAM line.



NUCLEASE QC

RNaseAlarm and DNaseAlarm
AU2042 • AU2056

Lateral flow activity assays that reveal RNase or DNase contamination in liquids and on surfaces.



BUFFERS

RNase-Free LFA Running Buffer Screening Pack
AU2035

Five 10 mL running buffer formulations, functionally tested in DNA- and RNA-based lateral flow.



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Universal LAMP-Optimized LFA Kit (Strep-Gold)
AU2073

2-, 3- and 4-line strips with LAMP-optimized RNase-free running buffer for multiplexed LAMP readout.



HYBRIDIZATION

On-Strip Hybridization NALF Starter Kit
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Biotin-streptavidin capture probes and anti-FITC/FAM gold for sequence-specific detection directly on the strip.



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Fluorescent Universal LFA Kits
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Streptavidin quantum dot, europium or R-phycoerythrin reporters for reader-based fluorescent detection.

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