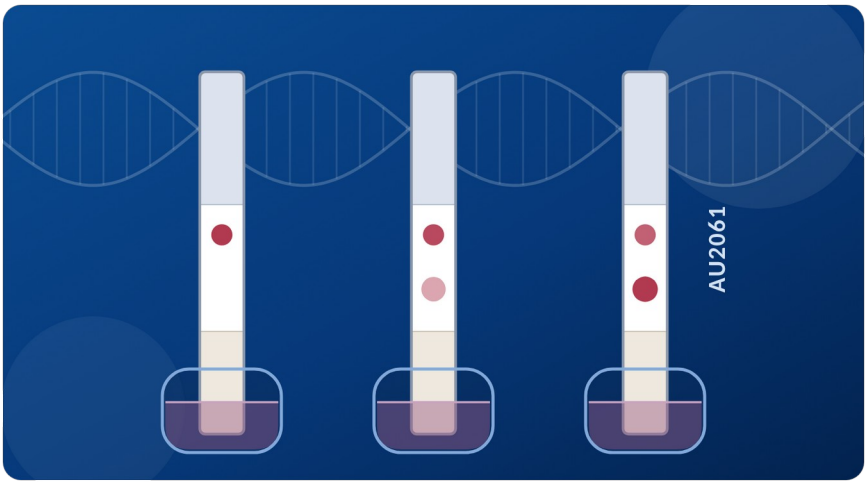


On-Strip Nucleic Acid Hybridization Lateral Flow Starter Kit

For Laboratory Use

Catalog Number: **AU2061**

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

1. Introduction

Attogene's On-Strip Nucleic Acid Hybridization Lateral Flow Starter Kit contains everything needed to start developing sequence-specific nucleic acid lateral flow assays without striping, laminating or cutting equipment. You spot your own capture oligonucleotide onto blank lateral flow strips with a pipette, then detect a FITC/FAM-labeled nucleic acid that hybridizes to it directly on the strip.

How it works. A biotin-labeled capture oligonucleotide is mixed with streptavidin and spotted onto the nitrocellulose membrane, where the streptavidin anchors it. A goat anti-mouse IgG (GAM) control is spotted above it. The strip is then run in RNase-free On-Strip Hybridization Buffer containing anti-FITC/FAM colloidal gold and the sample. A FITC/FAM-labeled nucleic acid that carries a sequence complementary to the capture oligonucleotide binds the gold and hybridizes at the test spot, which turns red-purple. Gold that passes the test spot is captured by the GAM control spot (Figure 1).

Controls included. The kit includes a matched control pair: a 5′ biotin capture oligonucleotide and its fully complementary 5′ 6-FAM hybridization oligonucleotide (76 nucleotides each, DNA). Use them to learn the spotting and running steps before you test your own probes, and as a positive control in every experiment.

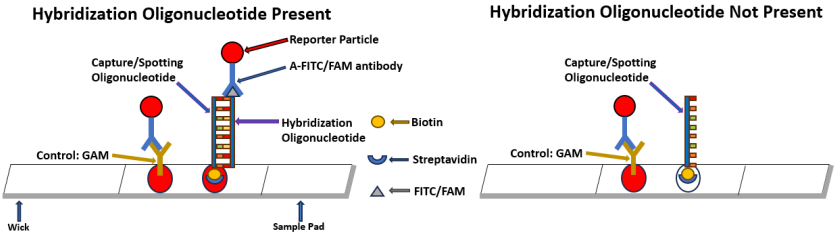


Figure 1. *Principle of on-strip hybridization (diagram). Left: the FITC/FAM-labeled hybridization oligonucleotide hybridizes to the biotin capture oligonucleotide, which is held on the membrane by streptavidin; anti-FITC/FAM gold binds the FAM label and the test spot develops. Right: without a complementary labeled nucleic acid only the GAM control spot develops.*

Features and benefits

- Develop sequence-specific (hybridization) lateral flow assays with a pipette; no striping, laminating or cutting equipment needed.
- Screen your own biotinylated capture probes and capture-probe-to-streptavidin ratios before committing to sprayed cards.
- Matched biotin and 6-FAM control oligonucleotides to verify every run.
- RNase-free hybridization buffer, compatible with DNA and RNA targets.

2. Kit Contents

Component	Amount	Storage
Blank 4.5 mm lateral flow strips (no lines)	100 strips, in two desiccated canisters	Room temperature, desiccated
Streptavidin, 10 mg/mL *about 200 pmol/µL*	10 µL	2–8 °C
Spotting Buffer	100 µL	2–8 °C
Control Capture (Spotting) Oligonucleotide *5′ biotin; 100 µM (100 pmol/µL)*	5 µL	2–8 °C
Control Hybridization Oligonucleotide *5′ 6-FAM; 1 µM (1 pmol/µL)*	100 µL	2–8 °C
Anti-FITC/FAM Colloidal Gold *15 nm*	1 mL	2–8 °C, protected from light; do not freeze
Goat Anti-Mouse IgG (GAM) control *ready to spot*	100 µL	2–8 °C
RNase-Free On-Strip Hybridization Buffer	15 mL	Room temperature
96-well plate + 4 extra wells	1	Room temperature
Manual	1	Room temperature

Packaging: the kit ships in one pouch. Inside are the blank strips in two desiccated canisters, the 96-well plate + 4 extra wells in its own pouch, the reagent tubes and the hybridization buffer bottle.

3. Product Specifications

Key specifications for experienced users.

Parameter	Specification
Format	Blank dipstick, 4.5 mm wide × 60 mm long; no lines striped; 100 strips per kit
Strip build	Glass fiber sample pad, nitrocellulose membrane and absorbent pad on an adhesive backing card (Figure 2)
Reporter	15 nm colloidal gold conjugated to anti-FITC/FAM monoclonal antibody; spots develop red-purple
Test spot	Streptavidin + biotin-labeled capture oligonucleotide (supplied control or your own), 1:1 molar ratio, 10–25 pmol of each per spot; 0.5–1 µL per spot
Control spot	Goat anti-mouse IgG (GAM), ready to spot; 0.5–1 µL per spot; captures gold that passes the test spot
Hybridization buffer	150 µL per test; 15 mL supplied (100 tests)
Gold	10 µL per test
Positive control	About 1 pmol of Control Hybridization Oligonucleotide per test (1 µL)
Time to result	Run and read at 30 minutes, unless your probe or application shows that a different time is optimal
Compatible targets	DNA or RNA carrying FITC/FAM and a single-stranded sequence complementary to the capture oligonucleotide, including labeled oligonucleotides and amplification products
Storage	Given per component (Section 4)

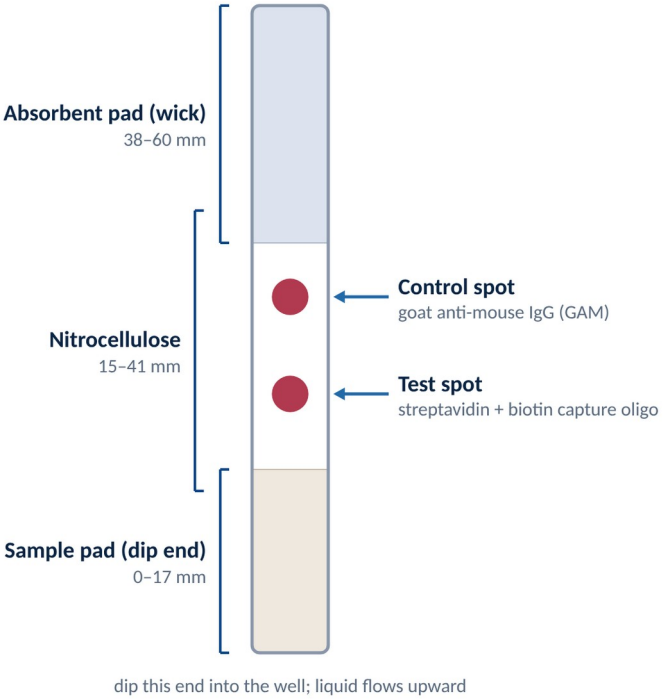


Figure 2. *Blank strip and where to spot (lengths to scale; strip width exaggerated; spot positions are illustrative). Spot the test mix on the lower part of the nitrocellulose, nearer the sample pad, and the GAM control on the upper part, nearer the absorbent pad. Dip the sample pad end into the well.*

4. Storage and Stability

- Store the blank strips, the On-Strip Hybridization Buffer and the 96-well plate at room temperature.
- Store the streptavidin, Spotting Buffer, control oligonucleotides, anti-FITC/FAM gold and GAM control refrigerated (2–8 °C).
- Protect the anti-FITC/FAM gold from light and do not freeze it. Freezing aggregates the gold irreversibly.
- Keep the blank strips in their desiccated canisters until use. Close the canister promptly after removing strips.
- Do not use the kit after the expiration date on the label.

5. Required Materials Not Supplied

- Pipette able to dispense 0.5–1 µL accurately (for spotting), with tips

- Pipettes and tips for 1–200 µL
- Incubator or oven at 37 °C (for drying spotted strips)
- Microcentrifuge (to collect the contents of the small tubes)
- Low-binding microcentrifuge or PCR tubes for spotting mixes
- Timer
- Optional: your own biotin-labeled capture oligonucleotides and FITC/FAM-labeled targets

6. Precautions

- Check the expiration date on the label before use.
- Handle strips by the edges or by the absorbent pad. Do not touch the nitrocellulose membrane with fingers or pipette tips.
- Use RNase-free tips and tubes when working with RNA, and a new tip for every reagent and sample to avoid cross-contamination.
- Keep amplified products away from the hybridization buffer, reagent stocks and unused strips.
- Use the components of one kit lot together.
- Include a positive control (control oligonucleotide pair) and a negative control (no hybridization oligonucleotide) in every experiment.
- The amount of labeled nucleic acid relative to the gold and the capture spot affects the result. If the test spot is weak at high sample input, run a serial dilution (hook effect).

7. Procedure

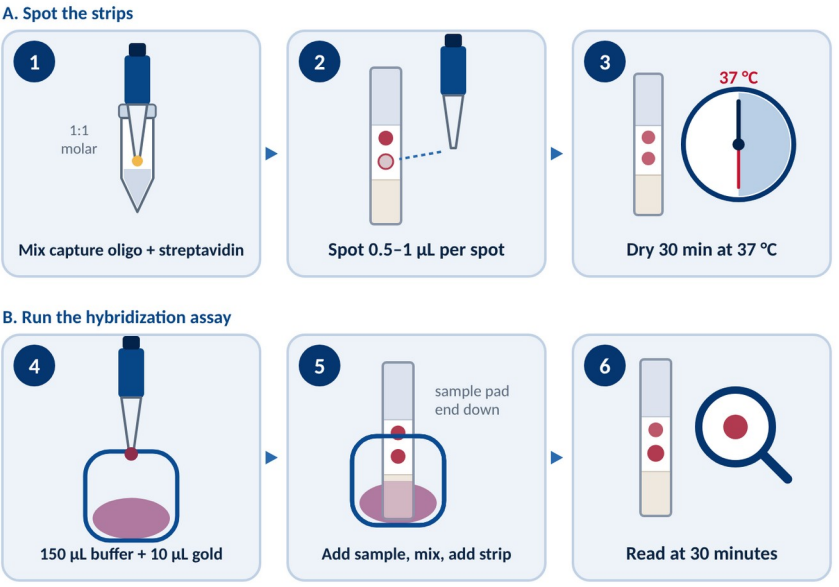


Figure 3. *Procedure at a glance. Steps 1–3 prepare spotted strips; steps 4–6 run the hybridization assay.*

7.1 Before you start

1. Spin all tubes briefly in a microcentrifuge to collect the contents at the bottom, especially the streptavidin and the Control Capture Oligonucleotide.
2. Bring the anti-FITC/FAM gold to room temperature and mix it well before use.
3. Resuspend your own biotin-labeled capture oligonucleotides at 100 µM (100 pmol/µL). This stock makes it easy to dilute into Spotting Buffer at different ratios.

7.2 Prepare the test spotting mix

4. Combine the biotin-labeled capture oligonucleotide and the streptavidin in Spotting Buffer at a 1:1 molar ratio, with 10–25 pmol of each in the volume you will spot (0.5–1 µL). Table 1 gives an example with the supplied control.

5. Mix by pipetting up and down.

Add, in this order	Volume	Amount
Spotting Buffer	7 µL	–
Streptavidin (10 mg/mL)	1 µL	about 200 pmol
Control Capture Oligonucleotide (100 µM)	2 µL	200 pmol
Total	**10 µL**	**20 pmol of each per µL**

Table 1. *Example test spotting mix. A 1 µL spot delivers 20 pmol of capture oligonucleotide and 20 pmol of streptavidin; a 0.5 µL spot delivers 10 pmol of each. To screen your own probe, keep the oligonucleotide amount constant and vary the streptavidin (for example 1:2, 1:1 and 2:1).*

7.3 Spot and dry the strips

6. Lay the blank strips flat on a clean, dry surface.
7. Apply 0.5–1 µL of the test spotting mix to the lower part of the nitrocellulose, nearer the sample pad (Figure 2). Touch the drop to the membrane; do not press the tip into it.
8. Apply 0.5–1 µL of the GAM control to the upper part of the nitrocellulose, nearer the absorbent pad. Leave enough space that the two spots do not touch.
9. Dry the spotted strips at 37 °C for 30 minutes.
10. Store the dried strips in a dry place, such as a desiccated canister, until use.

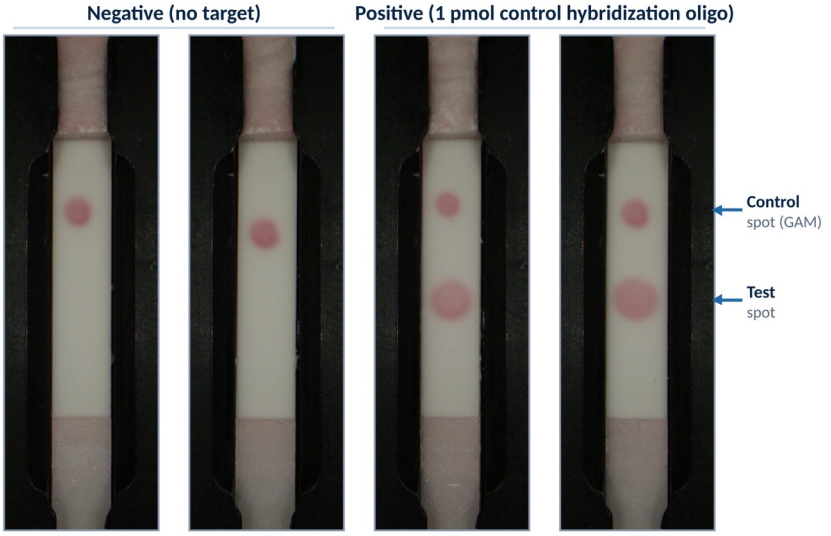
7.4 Run the hybridization assay

11. Add 150 µL of RNase-Free On-Strip Hybridization Buffer to a well of the 96-well plate. Prepare one well per strip.
12. Add 10 µL of anti-FITC/FAM gold to each well.
13. **Add the sample:**
    - **Positive control:** 1 µL of Control Hybridization Oligonucleotide (about 1 pmol), run on a strip spotted with the control capture mix.
    - **Sample:** your FITC/FAM-labeled nucleic acid or reaction mixture.
    - **Negative control:** nothing added (buffer and gold only).
14. Mix each well by pipetting up and down several times.
15. Insert one spotted strip into each well, sample pad end down.
16. Let the strip run for 30 minutes, then read it by eye, by photograph or with a lateral flow reader.

Note: these conditions are a starting point. The optimal amount of capture oligonucleotide, streptavidin ratio, gold volume and run time depend on your probe and target and may need optimization.

8. Interpretation of Results

- **Control spot:** must appear on every strip. A strip without a control spot did not run correctly; repeat the test with a new strip.
- **Test spot:** appears when a FITC/FAM-labeled nucleic acid hybridizes to the capture oligonucleotide. Within the working range, the test spot becomes stronger as the amount of labeled target increases. Compare every sample with the negative control strip.
- **Control spot intensity:** may weaken as the test spot strengthens, because gold captured at the test spot no longer reaches the control spot. This is expected.



Absorbent pad at the top; sample pad (dip end) at the bottom.
Kit release strips in a reader holder; photographs cropped, otherwise unedited.

Figure 4. *Example results (kit release test). Strips spotted with 20 pmol of streptavidin and 20 pmol of Control Capture Oligonucleotide (1:1) and with the GAM control; run in 150 µL of hybridization buffer with 10 µL of anti-FITC/FAM gold for 30 minutes, without (left) and with (right) 1 pmol of Control Hybridization Oligonucleotide.*

9. Designing Capture and Hybridization Oligonucleotides

A test spot needs two molecules that meet on the strip: a capture oligonucleotide held on the membrane, and a labeled nucleic acid that hybridizes to it and carries the gold.

Capture oligonucleotide (spotted)

- Must carry a biotin label, which binds the supplied streptavidin. Chemically synthesized oligonucleotides can be ordered with biotin at the position you need; the supplied control has a 5′ biotin.
- Its sequence must be complementary to a single-stranded region of the target.

Target or hybridization oligonucleotide (in the well)

- Must carry FITC/FAM, which binds the anti-FITC/FAM gold, and a single-stranded region complementary to the capture oligonucleotide. A molecule that lacks either one does not produce a test spot.
- Double-stranded amplification products must provide a single-stranded region that can hybridize to the capture oligonucleotide.

Control oligonucleotide pair (DNA, 76 nucleotides each)

Capture: 5′-biotin-CGTACGGAAT TCGCTAGCCC CCCGGCAGGC CACGGCTTGG GTTGGTCCCA CTGCGCGTGG ATCCGAGCTC CACGTG-3′

Hybridization: 5′-6-FAM-CACGTGGAGC TCGGATCCAC GCGCAGTGGG ACCAACCCAA GCCGTGGCCT GCCGGGGGGC TAGCGAATTC CGTACG-3′

The two sequences are fully complementary.

Controls and stoichiometry

- **Ratio screening:** the 1:1 molar ratio of capture oligonucleotide to streptavidin is a starting point. Screen other ratios with your own probe (Table 1).
- **Negative control:** always run a strip without hybridization oligonucleotide, and a no-template control for amplification products.
- **Excess target:** very high amounts of labeled nucleic acid can saturate the gold and weaken the test spot (hook effect). Dilute the sample in a series to find the working range.
- **Reference amount:** 1 pmol of Control Hybridization Oligonucleotide gives a clear test spot with the control capture spot (Figure 4).

10. Troubleshooting

Observation	Likely cause	What to do
No control spot	Strip inserted upside down, not enough buffer in the well, GAM not spotted or strip exposed to moisture	Insert the sample pad end down; use 150 µL of buffer; spot 0.5–1 µL of GAM and dry it fully; keep strips desiccated
No test spot with the control oligonucleotide pair	Capture mix not spotted, too little capture oligonucleotide, or gold not added	Repeat the spotting mix (Table 1); spot 0.5–1 µL; add 10 µL of gold to the well
Weak test spot with your own probe	Capture-to-streptavidin ratio not optimal, too little target, or target region not single-stranded	Screen ratios (Table 1); increase the amount of target; check the target design (Section 9)
Test spot weak at high sample input	Excess labeled nucleic acid (hook effect)	Run a serial dilution of the sample
Test spot in the negative control	Carry-over of labeled product, or the capture mix spread into the control area	Use a new tip for every well; leave space between spots; keep amplified products away from reagents
Spots spread, smeared or merged	Spot volume too large, or strips not fully dried before use	Spot 0.5–1 µL; dry at 37 °C for 30 minutes
Spots change after the read time	Strip read later than the read time	Read at 30 minutes, or at the time validated for your application

Spotting Buffer is supplied ready to use. In Attogene's testing, adding sucrose to the spotting mix weakened the test spot, so avoid additives unless you test them.

11. Additional Analysis

If necessary, results can be confirmed with a nucleic acid analysis technique such as agarose or polyacrylamide gel electrophoresis. A lateral flow reader can give numerical spot intensities.

12. Ordering Information

Catalog no.	Description	Size
AU2061	On-Strip Nucleic Acid Hybridization Lateral Flow Starter Kit	100 strips

Related products

Catalog no.	Description
AU2060	Anti-FITC/FAM Colloidal Gold (15 nm)
AU2034-04/-05/-06	Universal Lateral Flow Assay Kits, anti-FITC/FAM gold, 2-, 3- and 4-line
AU2063	FITC/FAM Nucleic Acid Stoichiometry QC Kit
AU2054	Blank 4.5 mm Lateral Flow Strips for LFA Development
AU2059	Lateral Flow Development Starter Kit

For sprayed capture probes on nitrocellulose cards, custom strips or other formats, contact sales@attogene.com or call 512-333-1330.

Contact Us

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Austin, TX 78744
Phone: 512-333-1330
Email: sales@attogene.com
Web: www.attogene.com

13. Quick Reference

Spotting at a glance

Step	Action
1	Mix capture oligonucleotide and streptavidin 1:1 in Spotting Buffer (10–25 pmol of each per spot).
2	Spot 0.5–1 µL of test mix low on the membrane and 0.5–1 µL of GAM above it.
3	Dry at 37 °C for 30 minutes; keep dry until use.

Running at a glance

Step	Action
4	Add 150 µL of hybridization buffer and 10 µL of gold to a well.
5	Add the sample. Positive control: 1 µL of Control Hybridization Oligonucleotide (about 1 pmol). Negative control: nothing added.
6	Mix by pipetting; insert the strip, sample pad end down.
7	Run and read at 30 minutes.

Reading the strip

- **Control spot:** must appear; no control spot means the strip did not run correctly.
- **Test spot:** compare with the negative control strip; a test spot means labeled target hybridized to the capture oligonucleotide.

Storage

- Strips, hybridization buffer and plate at room temperature; strips desiccated.
- Streptavidin, Spotting Buffer, oligonucleotides, gold and GAM at 2–8 °C; gold protected from light, never frozen.

Notes

Notes

RELATED PRODUCTS FROM ATTOGENE

Take your nucleic acid lateral flow further

Reagents, controls and development tools from the team that builds the On-Strip Hybridization Starter Kit.

SCALE UP

Anti-FITC/FAM Colloidal Gold

AU2060

15 nm gold, 10 OD, conjugated to anti-FITC/FAM, for your own lateral flow development or final product. Includes 50 mL RNase-free running buffer.

ASSAY QC

FITC/FAM Nucleic Acid Stoichiometry QC Kit

AU2063

Find the working range of your FAM-labeled product before full development. Oligo controls from 0.25 to 2.5 µM.

CONTROLS

FAM/Biotin 23S Amplicon Control DNA

NA2032

874 bp end-labeled PCR product (biotin forward, FAM reverse primer): a full-length amplicon control for the streptavidin line.

NUCLEASE QC

RNaseAlarm and DNaseAlarm

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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.

ISOTHERMAL

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.

READY-MADE STRIPS

Universal Lateral Flow Assay Kits

AU2034-04 · -05 · -06

2-, 3- and 4-line anti-FITC/FAM gold dipsticks with streptavidin, anti-Dig and anti-DNP test lines. No spotting needed.

FLUORESCENCE

Fluorescent Universal LFA Kits

AU2037 · AU2049 · AU2053

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

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- **LF Development Starter Kit** — AU2059
- **Oligo-BSA Conjugation Kit** — NA2034
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