

3913 Todd Lane Suite 310 Austin Texas 78744 On Strip Nucleic Acid Hybridization Lateral Flow Starter Kit Catalog Number: AU2061

Component	Amount	Storage
		Condition
Streptavidin (10mg/mL)	10ul at 10mg/mL – (200pmol/ul)	4°C
Spotting Buffer	100ul	4°C
Control Capture/Spotting Oligonucleotide 5/5Biosg/CGTACGGAATTCGCTACCCCCCGCAGGCCACG GCTTGGGTTGGTCCCACTGCCCCGCGAGGCCCACGT G/3	5ul at 100uM (100pmol/ul)	4°C
Control Hybridization Oligonucleotide 5/56FAM/CACGTGGAGCTCGGATCCACGCGCAGTGGGACCA ACCCAAGCCGTGGCCTCCGGGAGCCTAGCGAATTCCGTA CG3 ²	100ul at 1uM (1pmol/ul)	4ºC
Anti-FAM/FITC Colloidal Gold	1mL	4°C
RNase Free On-Strip Hybridization Buffer	15mL	RT
Goat-Anti mouse (Control)	100ul (ready to use formulation)	4°C
Blank Lateral Flow Strips	100	RT
96 Well Plate	1	RT

During lateral flow development, spotting reagents to the nitrocellulose strips is a convenient way to begin optimization and development studies. In this kit, we offer the components needed to perform development of "On Strip" Nucleic Acid Hybridization Techniques which allows researchers who do not have expensive lateral flow spraying, laminating and cutting equipment to perform much of their own upfront development work.

The kit has supplied capture and hybridization probes. The user can also include user supplied biotinylated capture and FAM/FITC hybridization probes for their evaluation. The kit also includes colloidal gold attached to anti-FITC/FAM antibody and "ready to spot" Goat Anti-Mouse control. The capture probe is then mixed with the supplied streptavidin in spotting buffer and the hybridization probe is applied to anti-FITC/FAM gold in "On-Strip" hybridization Buffer.

For more advanced "On Strip" nucleic acid hybridization methods and custom capture probe spraying onto nitrocellulose cards or questions about this kit, please contact for custom service offering at 512-333-1330 or by e-mail at sales@attogene.com.

To start, make sure all the solutions are quickly spun to bring the low volume liquids (streptavidin and Control Capture/Spotting Oligonucleotide) to the bottom of the tube.

Spotting Oligonucleotide:

To spot nucleic acid onto lateral flow strips using this kit, the capture probe must contain a biotin to associate with the provided streptavidin. Streptavidin enables the capture-oligonucleotide to



stay attached to the nitrocellulose. Chemically synthesized oligonucleotides can be purchased with this added biotin to the appropriate location to perform your own reagent testing.

Instructions: mix in a 1:1 starting ratio, 10-25pmoles of biotin labeled capture probe with 10-25pmoles of streptavidin dissolved in spotting buffer. Adjust the ratio of oligonucleotide and streptavidin to screen for optimal ratio of user specific biotinylated capture probe with streptavidin.

Apply 0.5-1ul per spot on the blank strip using a pipette suitable to handle this volume. Typically, a test spot is applied to the lower section of the blank strip and the control (GAM) is applied to the upper section of the blank strip as shown in the picture to the right.



NOTE: It is a good idea to suspend the biotinylated oligonucleotides at 100uM (100pmoles/ul) to provide yourself with the ability to dilute into spotting buffer at different ratios.

Control Antibody Spotting:

Spotting the Goat Anti Mouse (GAM) antibody: The GAM antibody is in a solution and concentration ready for spotting: Apply 0.5-1ul per spot on the blank strip using a pipette suitable to handle this volume. GAM will associate with the FAM/FICT antibody that is attached to the colloidal gold.

After the spots have been deposited, let the strips dry at 37°C for 30 minutes and store them in a dry place until ready to use.

Running The Strip:

To perform the on-strip hybridization reaction, mix 150ul of the On-strip hybridization Buffer into a well of the 96 well plate. Add roughly 1pmol of control hybridization oligonucleotide or reaction mixture to the well and 10ul of anti-FITC gold. Mix well and then add the appropriate spotted strip into the well and let it run for <u>30 minutes</u>. The "on-strip hybridization technique usually takes 30 minutes to run but this may vary depending on your hybridization probe and it may need optimization.

NOTE: The above testing is a starting point for performing "on strip" hybridization techniques. For more advanced on strip hybridization methods and custom capture probe spraying onto nitrocellulose cards please contact for custom service offering at 512-333-1330 or by e-mail at sales@attogene.com.