FITC/FAM Nucleic Acid Stoichiometry QC Kit

For Laboratory Use

Catalog Number: AU2063

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

1. Introduction

The fluorescein isothiocyanate (FITC) or 6-carboxyfluorescein (FAM) FITC/FAM Nucleic Acid Stoichiometry QC kit is configured with an anti-FITC/FAM colloidal gold conjugate embedded into the sample/conjugate pad at the bottom of the strip. The strip itself has a FAM-BSA conjugate to capture the anti-FITC/FAM colloidal gold. This kit is used to determine the stoichiometry of the FITC/FAM labeled reaction product is within range of the test. When a strip is introduced to the well with sample/standard and running buffer, the anti-FITC/FAM gold will flow through the nitrocellulose. Any anti-FITC/FAM gold (not associated with the FITC/FAM sample or standard) will be captured by the FAM-BSA line located on the center of the strip. Any anti-FITC/FAM gold (associated with the FITC/FAM sample or standard) will flow past the test line to the wick **(Figure 1)**.

AU2063 kit is a companion product to the AU2034-04/05 anti-FAM gold lateral flow strips, which have Goat Anti-Mouse (GAM) as the control line and Streptavidin as the test line (AU2034-04 kit only), and Goat Anti-Mouse as the control line, Anti-Dig for test line 2, and Streptavidin for test line 1 (AU2034-05 kit only).

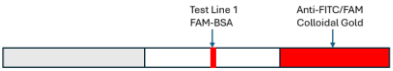
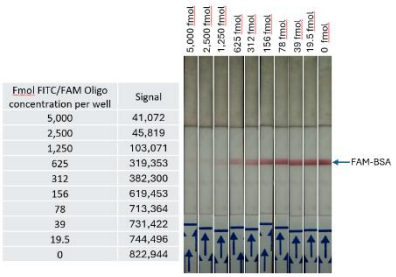


Figure 1. Demonstrates the configuration of the test strip.

In this kit (AU2063) the test line consists of FAM-BSA, which allows for the determination of analyte to gold stoichiometry in the lateral flow assay to make sure your sample is in range of the lateral flow architecture. The test can be used for quality control, ensuring that a given sample falls within the expected concentration range for proper downstream application.

Detection of nucleic acid (DNA or RNA) using this system requires the use of a fluorescein isothiocyanate or 6-carboxyfluorescein (FITC/FAM)-labelled nucleic acid.



Fmol FITC/FAM Oligo concentration per well	Signal
5,000	41,072
2,500	45,819
1,250	103,071
625	319,353
312	382,300
156	619,453
78	713,364
39	731,422
19.5	744,496
0	822,944

Figure 2. This data demonstrates the importance of the relative amounts of molecules of a synthetic FITC/FAM labeled oligo with the amount of anti-FITC/FAM gold particles to find optimal line intensities. The data shown above is only intended for demonstration purposes only. User provided nucleic acids will vary in performance and the provided figure should not be used for quantification of your product but as a guide.

The sample or standard containing FITC/FAM labeled nucleic acid is simply mixed into the specially designed nuclease free assay running buffer in a well of the supplied 96-well plate, and the dipstick is then added. Generally, the reaction is complete in 10-15 minutes. It is important to note that the relative stoichiometry between the FITC/FAM nucleic acid added, and the anti-FITC/FAM gold is important in assay optimization (**Figure 2**). As more FITC/FAM labeled nucleic acid products are added into the reaction, the test line will eventually

diminish as the analyte is competing for the same binding site on the anti-FITC/FAM gold conjugate as the test line (FAM-BSA). Therefore, a comparison of the sample should be made with a strip containing only sample running buffer in each experiment. The appropriate concentration of FITC/FAM labeled nucleic acid for use with the 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 labeled (FITC/FAM) nucleic acid that has a well characterized size, purity and concentration is included in the kit to compare with the sample.

Features & Benefits

- Can be used for the 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
3mm Dipsticks	50 each	RT
Nuclease Free Lateral Flow Running Buffer	10 mL	RT
Control Nucleic Acid containing biotin and FITC/FAM 0.5μM (2.5 picomoles/5μL)	20 μL	Refrigerate
Control Nucleic Acid containing biotin and FITC/FAM 0.2μM (1 picomole/5μL)	20 μL	Refrigerate
Control Nucleic Acid containing biotin and FITC/FAM 0.12μM (0.5 picomoles/5μL)	20 μL	Refrigerate
Control Nucleic Acid containing biotin and FITC/FAM 0.05μM (0.25 picomoles/5μL)	20 μL	Refrigerate
96 well plate	1 each	RT
Manual	1 each	RT

3. Storage and Stability

- The kit should be stored at 2°C - 30°C until ready to use.
- The test must remain in the sealed pouch until use.

4. Required Materials Not Supplied

- Timer - For timing use
- Centrifuge - For preparation of clear specimens

- Pipettor and pipette tips – to transfer samples and controls
- Molecules of interest containing FITC/FAM.
- Tubes or microtiter plates to run the strips

5. 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 test strips are packaged in a foil pouch with a desiccant.
- 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.
- 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 to see the test line.

7. Procedure

Perform the following:

1. Add 100µL 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 1µL-5µL are good starting points) of product into the sample running buffer. When running an 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 15µL if possible. <u>If larger amounts of sample need to be added, please inquire about our 2X nuclease free sample running buffers.</u>
   B. (POSITIVE CONTROL, 4 Strips Total) mix 5µL of each provided control oligonucleotide into their own respective well.
   C. (NEGATIVE CONTROL, 1 Strip Total) leave this devoid of any analyte (don't add any sample or control)
3. Mix each well completely by pipetting up and down several times.
4. Add one dip stick 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 the test line isn't present with your controls, or you don't observe a reduction in the test line as more of the control nucleic acid is added, ensure the kit isn't expired by checking the label.
NOTE: Control oligonucleotide line should eliminate the test line signal on the strip in a linear curve within 15 minutes at sufficiently high quantities.
NOTE: The data from this kit can be used to estimate the relative amount of sample to add into the sample running buffer when performing a test using kit AU2034-04 and AU2034-05.

8. Interpretation of Results

This test is a lateral flow assay containing test lines that are dependent on the concentration of FITC/FAM labeled nucleic acids in the sample. The provided controls are to be used only as a general reference and not to quantify the concentration of the user provided analyte. As the test is semi-quantitative, use quantitative methods such as acrylamide gel, HPLC, absorbance, fluorescent quantification etc. to provide accurate concentrations.

What to expect at the test lines:

Once the concentration of nucleic acid in the sample saturates the system a decrease in the intensity of test line will be observed - compared to the strip lacking nucleic acid (negative control strip).
Determination made using strips which have dried for a period outside of the required time may be inaccurate, as line intensities may vary with drying time.

9. Additional Analysis

If necessary, positive samples can be confirmed by analyzing using nucleic acid analysis techniques such as agarose or acrylamide gels. A lateral flow reader may also be employed to generate numerical readings.

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