Biotin Lateral Flow Assay Kit

Catalog Number: EL2021_LF

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

1. Introduction

Biotinylation is the process of labelling a molecule with a biotin molecule to take advantage of the strong binding and high specificity of biotin and streptavidin for purification or detection applications. Biotinylation is typically used to label proteins or nucleic acid-based targets for these applications. Quantitation of biotinylation can be used to optimize the labelling of a given target. Levels of biotinylation can affect detection sensitivity or functionality of a purification product.

This assay is based on the competition of biotin in the sample with streptavidin as a capture (test) line. A static control line is composed of a goat anti rabbit IgG is used for ensuring the test is run properly.

The sample containing the biotin to be detected is simply mixed into the specially designed 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 10-15 minutes.

2. Features and Benefits

- Easy method for detecting biotin in a sample.
- No expensive equipment required.
- Cost-effective way to screen for further downstream lateral flow assay development.

3. Kit Contents

Component Name	Volume	Storage
4.5mm Dipsticks	50 each	RT
Biotin Lateral Flow Running Buffer	10 mL	RT
D-Biotin control	20 µL	Refrigerate
96 well plate	1 each	RT
Manual	1 each	RT

4. 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.

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 either FITC (01 version) or DIG (02 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 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.
- 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 qdot and the test line capture reagents to see the test line.

7. Procedure

Perform the following:

1. Add 150µL of Biotin 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 Biotin Lateral Flow 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 15µL if possible.
    B. (POSITIVE CONTROL) mix 5µL of the control D biotin
    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 10-15 minutes
6. Visually analyze the strip by eye by shining with a black light or read in a fluorescent lateral flow reader. Specialized readers are needed to obtain the 2-100x increased levels of sensitivity described in the literature.

NOTE: If a higher analytical sensitivity is required, it could be helpful to increase the volume and/or concentration of the sample added into the well. Volume and concentration of analyte-specific solutions, and incubation time are always part of the individual test development.

8. Interpretation of Results

This test is a lateral flow assay containing a test line that is independent on the concentration of Biotin in the sample.

What to expect at the test lines:

The higher the concentration of Biotin in the sample the lower the intensity of the test line compared to the strip lacking biotin (negative control strip).

What to expect at the control line:

The intensity of the control should not change as a function of biotin concentration.

Determination made using strips which have dried for more or less than 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 a nucleic acid analysis technique such as agarose or acrylamide gels. A lateral flow reader may also be employed to generate numerical readings.

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