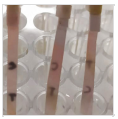

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Lateral flow buildup

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1 Works for me dx.doi.org/10.17504/protocols.io.8h2ht8e

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ABSTRACT

The following protocol describes how to build a half-strip simplified lateral flow sensor. Preparation of test strips, detection reagents immobilization and test development.

The purpose of this protocol is testing the proper functioning of the analytical mechanism of a lateral flow assay design. The strips have been simplified for fitting in 96-plate wells. The system doesn't integrate microfluidics for simplification of the assay.

GUIDELINES

Nitrocellulose membranes are delicated material which should be manipulated with gloved hands.

MATERIALS

NAME	CATALOG #	VENDOR
Whatman® FF80HP Din A4	FF80HP DIN A4 10/pk	

MATERIALS TEXT

- Streptavidin 5 µg/µL suspension in PBS
- 5' Amino terminal modified test aptamer at 100 µM cocnentration in distilled water.
- 5' Amino terminal modified control DNA sequence (18x Poly Thymine tail).
- PBS pH = 7.4 buffer
- Cellulose Absorbent Pad

- 0.2-2 µL Micropipette
- 10µL Micropipette tips
- Laboratory paper tape
- Eppendorf Tubes (1.5 mL)
- Thermoblock
- Ice
- Laboratory dissecator
- Untreated 96-well plate.

- E. Coli DH5α suspension in PBS 1.4 mM $MgCl_2$ OD600 = 0.3
- Aptamer conjugated latex beads suspension at 1% in PBS-T (0.05%)
- 0.2 % BSA solution in PBS

BEFORE STARTING

Before start assure fresh ice on an adequate container and preheating a thermoblock to 95 ºC.

Strips preparation

1 Cut 4 pieces of 5mm wide x 40 mm long strips from the nitrocellulose FF80HP membrane. Label in the plastic backing of the membranes T or C where the Control or Test dots will be deposited. The dots will be positioned at 15 mm and 25 mm from the bottom of the strip. Alternating their order in two of the strips (T/C in two membranes and C/T in the other two).

2 Cut 4 pieces of the cellulose absorbent pad 6mm wide x 12 mm long, and fix them with laboratory paper tape at the top of the strips, overlapping by 6 mm with the membrane. Assure that the tape makes soft pressure, allowing contact between absorbent pad and the membrane.

Reagents preparation

3 Pipette on two different 1.5 mL eppendorf tubes 10 µL of each aptamer suspensions. Put the tubes in a 95 ºC pre-heated thermoblock. Keep the tubes at 95ºC for 10 minutes to allow DNA to denaturalize.

4 After the 10 minutes, remove the tubes from the thermoblock, and place them quickly on ice. Let them chill for other 10 minutes. That will allow the DNA to acquire the most favorable thermodynamic conformation.

5 Mix on the previous eppendorf tube 10 µL of 100 µM suspension of amino terminal modified DNA sequence, with 10 µL of the 5 µg/µL streptavidin suspension.

Repeat that step for both DNA sequence to immobilize on the membrane and label the tubes.

Incubate the tubes in ice for 1 hour.

6 Prepare two additional eppendorf tubes and make a 1:6 dilution, taking 2 µL of each Streptavidin-DNA conjugate tubes and diluting in 10 µL of PBS buffer.

Reagents Immobilization

7 Place the strips horizontally on a flat surface and label them. A pair of them with different T/C region disposition will hold the 1:6 dilution (8 µM) of the reagents, the other one will be treated with the 50 µM prepared suspension.

8 Take 0.33 µL of the correspondant DNA dilution and pipette it very slow on the proper T/C region of the prepared strips. Repeat this step with each one of the four different tubes of streptavidin-biotinylated DNA conjugates.

Allow the membranes to dry for aproximately 15 minutes, and repeat three times more the reagents deposition, pipetting exactly in the same point as the previous time.

At the end 1.2 µL will have been deposited in each region.

9 Block the prepared membranes by immersion in 0.2 % BSA suspension for 30 seconds.

10 After the last deposition of reagents, let the membrane to completely dry, keeping it overnight at 4ºC in a dessicator.

Strip Test

11 Mix 200 µL of 1% wt aptamer conjugated beads with 400 µL of E. Coli prepared suspension previously mentioned in required materials. Incubate for 1 hour at room temperature.

12 Pipette 125 µL of the E. Coli - Aptamer conjugated beads in in 4 wells of the 96-plate. (125 µL per well, 500 in total).

13 Take each previously prepared membrane and fit it into the wells of the 96-plate. Allow sample migration for 30 minutes, letting it to get absorbed into the top absorbent pad.

14 Results can be directly visualized as visible colored spots on the membrane.