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Streptavidin immobilization stress test V.2

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ABSTRACT

The following protocol was performed to test the affinity of the protein streptavidin for the nitrocellulose when applied a continuous flow of solvent.

MATERIALS

NAME	CATALOG #	VENDOR
nitrocellulose membrane sheets size 210 m × 297 mm thickness 200 μm	Whatman® FF170HP Din A	Sigma Aldrich
Whatman® FF80HP Din A4	FF80HP DIN A4 10/pk	

MATERIALS TEXT

Reagents:

- Ponceau 0.1% with in acetic acid 5%
- PBS-tween 0.1%
- Streptavidin 5 μm/ml
- dH₂O

Materials:

- Micropipette 0.2-2 ul
- Tape
- Desiccator
- Jar

BEFORE STARTING

Make sure that the surfaces and materials are clean before start.

- 1 Cut the FF80HP nitrocellulose membranes in 2 rectangles of 0.8cm x 4 cm dimensions. Repeat this procedure with the FF170HP nitrocellulose to obtain a total of 4 nitrocellulose pieces.
- 2 Stamp the microfluidic design, following the protocol microfluidic channels wax priming on:
 - One piece of the FF170HP nitrocellulose membrane
 - One piece of the FF80HP nitrocellulose membrane.
- 3 Glue the absorbant pad with tape at the top edge top of the 4 pieces of nitrocellulose. the dimensions of the absorbant pad are 0.8 cm x 1.2 cm.

- 4 Deposit 0,33 ul of streptavidin solution with a micropipette. Repeat this procedure 3 times, letting the drop dry in between each time.
The procedure must be done in the 4 pieces of nitrocellulose.
- 5 Let the pieces dry overnight at 4°C in a desiccator.
- 6 Perform a thin-layer chromatography with PBS-Tween as the solvent: pour 15 ml of the solvent and position the pieces with the absorbant touching the bottom of the container.

Let the solvent flow trough the nitrocellulose for 15 min.

The assay can be done individually or with the 4 pieces of nitrocellulose at one time.
- 7 Let it dry at room temperature for 40 min
- 8 Reveal with Ponceau 0.1% within acetic acid 5% by immersing the nitrocellulose piece into the solution for a few seconds.
- 9 Destain in dH₂O in agitation for 2 min.



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