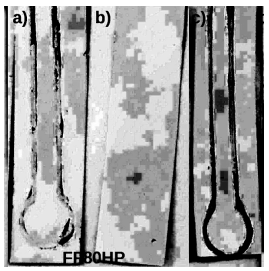
Oct 20, 2019 Version 1

Streptavidin immovilization stressed test V.1



DOI
dx.doi.org/10.17504/protocols.io.8hzht76

Jorge Fernández[1]
[1]Universidad Complutense de Madrid

AEGIS - Madrid iGEM 2019

Laura Armero

DOI: https://dx.doi.org/10.17504/protocols.io.8hzht76

Protocol Citation: Jorge Fernández 2019. Streptavidin immovilization stressed test. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.8hzht76**

Protocol status: Working
We use this protocol and it's working

Created: October 20, 2019

Last Modified: October 20, 2019

Protocol Integer ID: 28953

Keywords: Jorge Fernández, streptavidin immovilization, streptavidin, affinity of the protein, nitrocellulose, continuous flow of solvent, solvent, protein

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

MATERIALS

nitrocellulose membrane sheets size 210 m × 297 mm thickness 200 µm **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Whatman® FF170HP Din A

Whatman® FF80HP Din A4 **Catalog #**FF80HP DIN A4 10/pk

Reagents:
- Ponceau 0.1% with in acetic acid 5%
- PBS-tween 0.1%
-Streptavidin 5 um/ml
- dH_2O

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

Troubleshooting

Before start

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 4ºC 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 dH_2O in agitation for 2 min.