Oct 19, 2019 Version 1

# Quantitative paper ELONA V.1

DOI
dx.doi.org/10.17504/protocols.io.7arhid6

Manuela De Las Casas[1]

[1]Universidad Complutense de Madrid, AEGIS - Madrid iGEM 2019

AEGIS - Madrid iGEM 2019

Manuela De Las Casas
Universidad Complutense de Madrid, AEGIS - Madrid iGEM 2019

OPEN ACCESS

**DOI: dx.doi.org/10.17504/protocols.io.7arhid6**

**Protocol Citation:** Manuela De Las Casas 2019. Quantitative paper ELONA. **protocols.io https://dx.doi.org/10.17504/protocols.io.7arhid6**



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

**Created:** September 12, 2019

**Last Modified:** October 19, 2019

**Protocol Integer ID:** 27697

## Abstract

The aim of this protocol is to check the efficiency of the detection system that will be present in the strips. Like the qualitative assay, this is a dot blot performed with streptavidin and aptamers, also named paper ELONA. The expected result is a good binding between the streptavidin and the aptamers (knowing already that the streptavidin binds correctly to the nitrocellulose membrane thanks to previous experiments), great hybridization between both aptamers and a clear visible result when yielding with the ABTS. In order to make this experiment quantitative, several concentrations are made from the same mix, to test the minimum concentration with a detectable change of color.

## Guidelines

After washing the membranes with BSA or PBS, is convinient to let the membrane dry off for a little bit. Although it is not necessary to have it completely dry, if it's too wet, the drop of the next reagent added will probably elute out of the wax dot, which is inconvenient. If it's dry enough, the drop added will have enough surface tension to stay within the wax circle, which is the expected.

On the other hand, make sure when adding the antibody that the membrane will remain wet during the whole incubation time. Same with the ABTS. A poor control of these conditions will lead to less reproducible results.

## Materials

MATERIALS

Hot/Stir Plate

Pipette Tips

Glass Petri dishes 90 × 15 cm

Streptavidin, 1mg **Promega** **Catalog #**Z7041

BSA

Anti-Digoxigenin-AP, Fab fragments **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**11093274910

PBS

Eppendorf tubes 1.5 mL uncolored **Eppendorf** **Catalog #**022363204

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

Wax crayons (non water-soluble)

Ultrospec 1100 pro

ABTS solution **Roche** **Catalog #**11684302001

- Various pipettes in a range from 2µL to 1000µL.
- Wet paper to set the humid chamber.
- Ice.
- Tweezers
- Pen with permanent ink to mark the membranes.

## Safety warnings

Lab coat and gloves should be weared throughout the whole experiment. All working surfaces must be clean and all the reactives should be treated following manufacturer's instructions.

## Preparing the nitrocellulose strip

1

Set the hot plate to 100ºC or at least warm enough to melt the wax. Cut a small amount from one of the wax pencils and place it on a Petri dish. Set the Petri dish on the hot plate and wait for the wax to melt.

Cut a strip from the nitrocellulose sheet with the desired size.

Once the wax is melt, grab a 200µL pipette tip from the pointy side and place the broad end in the wax. Check the whole circle contains enough wax and carefully place it on the nitrocellulose strip. Apply a little pressure and remove the pipette tip. You should be able to see the wax circle on the strip. Repeat as many times as necessary to set all the dots for the assay.

Cover the nitrocellulose strip with paper (one sheet on top, another underneath). Place the sandwich on the hot place with a Petri dish on top (this is just to apply some pressure, any temperature-resistant flat object is valid here). Allow the strip to warm for 5 minutes. This will allow the wax to penetrate all through the strip.
Remove the strip from the hot plate and let it cool down again. You should see a circle of wax but the center must be clear, otherwise the protein will not have anywhere to bind. If the circle is too thick, consider repeating these steps.

## Hybridization of the aptamers and addition of the streptavidin

2 In an Eppendorf tube, mix the two aptamers selected: the aptamer Apta-Eco which has a biotin tag, and the reverse complementary aptamer to Apta-Eco marked with a digoxigenin tag. Check the concentrations are the same for both aptamers. We worked with the resuspended stock, which concentration was 100 µM. Then, add Aptamer Buffer (PBS, 1,4 mM $Mg^{2+}$) in proportion 1:1:3 with the aptamers.

From this mix, make serial dilutions. We got 6 different tubes with the dilutions 1, 1:2, 1:5, 1:10, 1:20, 1:50 to get a quantitative result.

2.1 Set the hot plate to 95ºC and place the tube with the mix for 5 minutes. After, put the tube back in ice to allow it to cool down again.

When the mix is cooled, mix it in proportion 1:1 with streptavidin 0,2 µg/µL (18µM). Let the protein and the aptamer bond for a few minutes in ice.

2.2 Finally, pipette 5 µL from the mix tube in each one of the wax dots previously prepared on the nitrocellulose membrane, and allow it to dry completely. It is recommended to set

at least 2 or 3 dots for each concentration, to increment the total n and have average data for each concentration.

## Setting the control dots

3 Prepare three different controls:

3.1 **NEGATIVE 1**. Pipette 5 µL of streptavidin 0,1 µg/mL on the dots reserved for this section. Allow it to dry. Without aptamers, this strip should not give a positive result. This one will not have antibody.

3.2 **NEGATIVE 2**. Pipette 5 µL of streptavidin 0,1 µg/mL on the dots reserved for this section. Allow it to dry. Similar to the negative 1, without aptamers, this strip should not give a positive result. But in this case, antibody will be added in further steps, to check if the presence of the aptamers is necessary for the reaction to happen.

3.3 **POSITIVE 3**. Pipette 5 µL of streptavidin 0,1 µg/mL on the dots reserved for this section. Allow it to dry. Add 5 µL of the antibody (Anti-Dig-POD 1:100 in PBS 1,4mM $Mg^{2+}$ BSA 0,2%). This control will reveal if the antibody by itself works when the free pores in the membrane are not blocked and the antibody binds non-specifically.

## Washing and blocking with BSA

4 Set the strip in a Petri dish and pipette BSA 5% (in PBS). Pipette excess of it and with tweezers, move the strip around gently, to let it wash and block the free spaces in the membrane. Take the strip out of the Petri dish and allow it to dry for a bit. It is not necessary that the strip is perfectly dry, but it must not be soaked. The more dry, the more chances for the antibody not to spread out of the wax dot.

## Addition of the antibody

5 Once is dry enough, pipette 5µL of the antibody (Anti-Dig-POD 1:100 in PBS 1,4mM $Mg^{2+}$ BSA 0,2%).

5.1 Incubate for one hour: set a humid chamber. This can be easily done by adding wet paper in a Petri dish. This will prevent the antibody from drying completely while incubating.

5.2 After one hour, remove the strips from the chamber, and wash them again with PBS (pipetting excessive liquid and moving gently with the help of tweezers. Allow it to dry enough to proceed to the next step (just like before, there is no need to completely dry the membrane).

## Reveal with ABTS

6

Set again the humid chamber and pipette 10 µL of ABTS on each dot. Incubate in humid conditions for 30 minutes. If no color is developed, 15 more minutes can be added. On the other hand, if the reaction happens fast and you are able to see a strong change in color, this period can be shortened to 15 minutes.

6.1

Dry the strips on the hot plate. 5 minutes at 95ºC should be enough. This will stop the reaction in all the strips at the same time, to make the results comparable. Independently of the total incubation duration, it is crucial that all the strips stop the reaction at the same time (otherwise, the results are not comparable).

## Results

7

To see the results with higher quality, they can be scanned. The density of each dot can be analysed with software tools.