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Streptavidin yielding with Ponceau

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Protocol status: In development

We are still developing and optimizing this protocol

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Abstract

The aim is to see if the streptavidin binds correctly to the nitrocelullose membrane. Only after achieving a positive result is recommended to proceed to the next step (the dot blot).



Materials

MATERIALS

☒ Hot/Stir Plate

☒ Pipette Tips

☒ Glass Petri dishes 90 × 15 cm

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

☒ Ponceau S **Gold Biotechnology Catalog #P-330**

☒ PBS

☒ 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)

🕒 30:00:00

🔥 Room temperature the hot plate must be warm enough to melt the wax. The streptavidin must be kept on ice to prevent degradation.

Safety information

Use disposable gloves and lab coat during the whole protocol.

Safety warnings

❗ Use gloves and lab coat. The Ponceau stain is hard to remove from surfaces, so a filter paper is adequate to use as protective layer.

Before start

Be sure you have a clean working surface. Be sure all the materials that will be in contact with the nitrocellulose are clean (you can clean the scissors and tweezers with alcohol).



Preparing the nitrocellulose strip

1

Set the hot plate to 100°C 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.

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 plat 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.

Adding the streptavidin

2 Pipette 5 µL of Streptavidin. If the protein is too diluted, you might not see a staining with the Ponceau. We ended using the protein suspended in PBS but without any dilutions.

Allow it to dry completely. Room temperature is adequate.

Ponceau yielding

3 Set the strip on a Petri dish and let it soak completely in Ponceau stain. Wash it repeatedly with water until the edges of the strip (where is no protein) are clear. Let it dry.

A positive result should show a difference in color between inside and outside the wax circle. The more concentrated the protein, the more intense should be the staining.