Overview

This document explains the materials necessary and the steps to perform a ponceau scan of a western blot.

Additional resources

Need more help?

Check the resources, and then see Ken

Main content

**Materials**

* + - Ponceau Stain

**Methods**

1. After scanning the membrane upstairs, download into Odyssey and make sure the scan worked. Only then begin staining with ponceau.
2. Pour enough Ponceau S staining solution to cover the membrane and place on the shaker at medium speed for 45 minutes.
3. Pour out the staining solution and spray with DI water to remove the stain. Continue until most of the red has disappeared on the surrounding area except for on the bands of protein.
4. Place membrane on a blotting paper in a drawer in order to dry before scanning.
5. Open Epson scan and verify the mode is set to Full Auto Mode.
6. Click Customize>File Save Settings.
7. Label the file exactly how it is labeled in Odyssey with “ponceau” at the end
8. Select the file to be saved as a Tiff and click OK and then Scan.
9. Edit the Tiff file in Photoshop. Under the image tab crop the image to only show the membrane and rotate if necessary. Click mode in the image tab and save it as a 16-bit, gray-scale image.
10. Be sure to save original sane and altered scan (labeled the same with “16 bitat the end) in the correct project folder.
11. Open Odyssey, go to File and click on Import image.
12. Label the file exactly how it is labeled in Odyssey with “ponceau” at the end.
13. Uses the Analysis name write Ponceau.
14. Use the 800 channel and browse for the 16-bit, gray scale image in the project folder it was saved to, then click OK
15. Go to alter image display under View. Click linear manual
16. Go to adjust image curves under View. Select grayscale. Click the boc that says inverse gray scal and adjust the curve.
17. Follow the Analyzing Scans in Odyssey (for westerns, ponceaus stains, and coomassie stains)protocol for the next step.