Wallert and Provost Lab

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Protocol for Ponseau S Staining

INTRODUCTION Ponceau S Staining Solution is used for the detection of proteins on cellulose acetate, PVDF, and nitrocellulose membranes.

For PVDF and nitrocellulose membranes, microgram quantities of transferred protein can be detected with a clear background and red protein bands. This staining technique is reversible to allow further immunological detection. The limit of detection for this stain is 250 nanograms of protein after separation by electrophoresis in polyacrylamide gels and transferred to nitrocellulose membranes (as described in Anal. Biochem., 156, 341-347 (1986).

Ponceau S is a negative stain which binds to the positively charged amino groups of the protein. It also binds non-covalently to non-polar regions in the protein. (Note: Ponceau S is not suitable for use with nylon membranes.)

PREPARATION OF PONCEAU S STAIN (Ponceau S: 0.1% (x/v) Ponceau S in 1% (v/v) acetic acid)

Add in order listed

- 10 ml MiliQ Water
- 0.3 ml glacial acetic acid (Do not use pipetman)
- 0.033 g Ponceau S
- QS to 30 ml with MiliQ water

Store at room temp.

PROCEDURE FOR TOTAL PROTEIN DETECTION:

1. After electrophoresis, immerse the blotted membrane in a sufficient amount of Ponceau S Staining Solution and stain for 5 minutes. DO THIS BEFORE BLOCKING

2. After staining, immerse the membrane in an aqueous solution containing 5% acetic acid (v/v) for 5 minutes, change the aqueous solution, and immerse the membrane for another 5 minutes.

3. Transfer the membrane into water for two washes of 5 minutes each.

4. Remove the membrane and block as normal.