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ProductInformation

PONCEAU S STAINING SOLUTION
Sigma Prod. No. P 7170

For Cellulose Acetate Protein Detection and Western Blot Total Protein Detection

USAGE:

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.)

Ponceau S Staining Solution contains 0.1% Ponceau S (w/v) and 5.0% acetic Acid (w/v). Store at room temperature. The product is at working concentration, to be used as sold without further dilution.

PROCEDURE FOR CELLULOSE ACETATE TOTAL PROTEIN DETECTION:

1. After electrophoresis, immerse the cellulose acetate membrane in a sufficient amount of Ponceau S Staining Solution and stain for 5 minutes.
2. After staining, immerse the membrane in an aqueous solution containing 10% acetic acid (v/v) for 5 minutes, change the aqueous solution, and immerse the membrane for another 5 minutes.
3. Transfer the membrane into methanol and leave it to soak for 5 minutes.
4. Finally, transfer the membrane into a clearing solution for 5 minutes. (Clearing solution contains methanol:acetic acid:polyethylene glycol MW 400 [PEG] in ratio by volume 70:30:4. (PEG P3265)
5. Remove the membrane form the clearing solution and dry it under a heating fan.

PROCEDURE FOR PVDF AND NITROCELLULOSE MEMBRANES:

Total Protein Detection:

1. After transferring proteins from SDS-PAGE onto membrane, immerse the membrane in sufficient Ponceau S Staining Solution and stain for 5 minutes.
2. Rinse membrane with distilled water until the background is clear.
3. Dry the membrane under a heating fan.

Reversible Protein Detection:

1. After proteins are transferred onto membrane, immerse the membrane in Ponceau S Staining Solution and stain for 5 minutes.
2. After proteins have been visualized, rinse the membrane with distilled water and rapidly immerse in an aqueous solution of 0.1 M NaOH. Protein bands will start to disappear after 10-30 seconds.
3. Rinse membrane with running distilled water for 2-3 minutes.
4. Continue with other procedure being followed (e.g. immunological detection).

ADDITIONAL NOTES ON USAGE:

A. The product can be used on most membranes including PVDF (polyvinylidene difluoride) except nylon membranes. Ponceau S does not destain from nylon membranes because the nylon is positively charged, the stain is negatively charged, and therefore, the stain is electrostatically held. Other membranes, like nitrocellulose, cellulose acetate, or PVDF are electrically neutral, and therefore, can easily be destained. For reversible staining of nylon membranes, a collodial iron procedure can be used (See *Anal. Biochem.*, Vol. 153, 18 (1986)).

B. A quick water rinse allows one to see if bands are developing, but destaining is usually done in 10% acetic acid. For sequencing or immunoassay purposes, the bands can be destained to colorless using 0.1 M NaOH.

C. If using nitrocellulose membranes, do not use alcohols in destaining procedure. For a western blot, rinsing the membrane with deionized water will remove any background dye. To remove the dye from the protein for further immunochemical testing, a rinse the membrane with 0.1 M NaOH as described above. Destaining in acetic acid is not done on the nitrocellulose membrane.