

Reversible staining of blots- Ponceau S

Ponceau S solution

0.1% Ponceau S - Sigma P 7767
5% glacial acetic acid
in aqueous solution

General Principle

Ponceau S is a red dye and will stick to proteins in preference to the membrane. The dye in low concentration will gradually wash away, but take longer to wash away from protein containing areas than from membrane alone. The retention time will depend heavily on pH; low pH regions will retain the dye longer.

- 1) Wash blot with ddH₂O 3x5 min
- 2) Optional: wash once with 5% Acetic acid for 2min
- 3) Drain water off of blot
- 4) Cover blot with Ponceau S solution
- 5) Agitate for about 30 seconds
- 6) Save Ponceau S solution (can reuse)
- 7) Wash with ddH₂O, watch blot, when bands become visible, photocopy or photograph.
- 8) Continue washing with ddH₂O to remove remaining Ponceau S.
-If some remains it should disappear when Western Blotting, blocking with TBST + 2% dry milk should get rid of remaining Ponceau S.