

Protocol: Ponceau Stain

Application:

Staining protein on PVDF or nitrocellulose membranes post-transfer (i.e. Western blotting) for the purpose of protein transfer verification.

Recipe:

Reagent	Amount	Final Concentration
H ₂ O	950 mL	XXX
Glacial Acetic Acid*	50 mL	5% vol/vol
Ponceau Red dye	1 gram	0.1% wt/vol
Total volume of solution**	1000 mL	

Caution: *Open glacial acetic acid in chemical fume hood.

**Store final solution at 4 °C.

Procedure:

1. Post-transfer, place membrane into flat-bottom container (protein side up).
2. Completely submerge with Ponceau stain and put on rocker
 - Nitrocellulose membranes saturate in ~5 min.
 - PVDF membranes saturate in ~15 minutes.
3. Remove Ponceau from non-protein parts of the membrane via gentle rinsing with distilled H₂O.
4. Capture image to verify blot quality.
5. Mark molecular weight standards and lanes to configure blot using **permanent** lab marker.
6. Completely remove Ponceau with 4-5x room temperature washes (~5-10 minutes per wash)
 - Using 1x TBS/0.2% Tween-20.
7. Once all the “red” is gone, membrane can be blocked and probed as per normal procedure.