

SOP SP051:

Ponceau-S Staining of Nitrocellulose Membranes

Materials and Reagents:

1. Concentrated Ponceau-S solution (note 1)
2. Nitrocellulose from Western Blot

Protocol:

1. ___ Dilute concentrated Ponceau-S solution 1:10 in MilliQ H₂O.
2. ___ After removing Nitrocellulose membrane from Transfer chamber SOP# SP011.1, place into diluted Ponceau-S solution and incubate at room temperature on rocking platform for 10 minutes.
3. ___ Wash with ddH₂O three times.
4. ___ Proteins should be visible as pink bands. The lanes/proteins can be marked with a pencil for future reference.
5. ___ Nitrocellulose membrane is now ready for Western blot blocking. There is no need to destain further since the pink dye will dissipate during the blocking step.

Notes:

1. Concentrated Ponceau-S Solution
2% (2mL) Ponceau-S = (3-hydroxy-4-[2-sulfo-4-(sulfo-phenylazo)phenylazo]-
2,7-naphthalene disulfonic acid)
30% (30mL) trichloroacetic acid
30% (30mL) sulfosalicylic acid
q.s. to 100mL with MilliQ H₂O
Stable at room temperature for 1 year

References:

Antibodies: A Laboratory Manual. Ed Harlow and David Lane. Cold Spring Harbor Laboratory, New York. 1988. pp. 492-494.