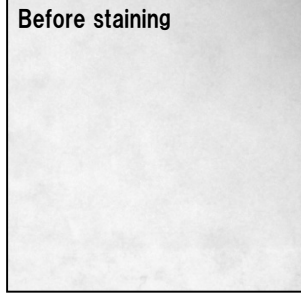


【Protocol】

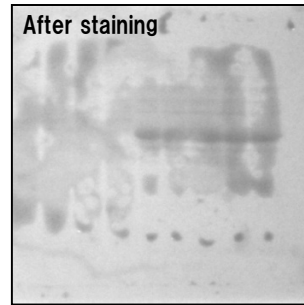
1. Removal of membranes

Remove membranes from blotting machine.



2. Staining

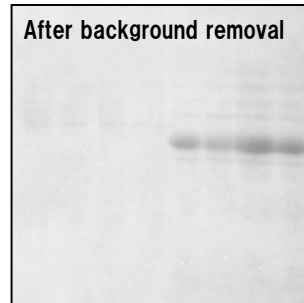
Put the membrane into plastic container, and pour Ponceau-S staining solution so that membrane is well soaked in the staining solution. Shake the container for 2 to 5 min to get the membrane stained.



3. Decolorization of background

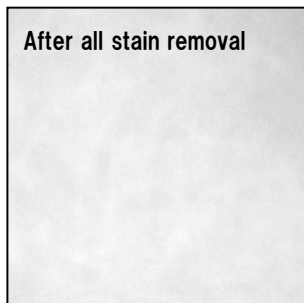
Discard the staining solution from the plastic container, and add 1% acetic acid solution so that the membrane is well soaked. Shake the container until the background staining get low enough so that the protein band get clear. Generally 2 min is enough for this process. (when taking pictures, take out the membrane form the container and place it on a wet paper filter. Be careful not to get the membrane dry.)

After removal of background staining and picture taking, Discard the acetic acid solution from the container and pour enough amount of DW to the container.



4. All removal of ponceau-S stain

Discard the DW from the container, and pour 0.1 M NaOH solution (dissolve NaOH 4g to 1L DW) so that the membrane is completely soaked. By checking the color of membrane, shake the container until to achieve complete removal of staining dye. Usually 10 to 20 sec is enough.



5. Washing

Discard the solution from the container, and pour enough volume of DW and shake the container. Repeat the same process again.

Go to next step for western blot (blocking).