Protocol

AzureRed Total Protein Membrane Staining

Preparation of Solutions

Before staining, prepare Fix, Stain, and Wash solutions as described below. These solutions are stable for up to 1 year when stored at room temperature. Precipitates or dust present in the solutions will result in speckling on gels. If observed, filter solutions before use. The amount of reagents in each packet of AzureRed Powder A or B is sufficient to prepare 1 L of solution. Do not split the packets. Once a packet is opened, the entire contents should be used. For preparation of larger volumes, use more than one packet.

Fix Solution

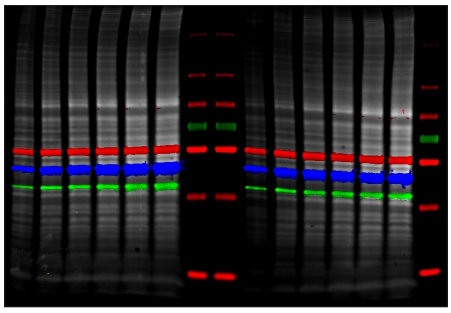
Add contents of one AzureRed Powder A packet (10.1 g) to 850 ml of high-purity water in a 1 L bottle. Mix until dissolved. Add 150 ml 100% ethanol and mix thoroughly.

Stain Buffer

Add contents of one AzureRed Powder B packet (23.4 g) to 1 L of high-purity water in a 1 L bottle. Mix until completely dissolved.

Wash Solution

Mix 850 ml high-purity water and 150 ml 100% ethanol in a 1 L bottle.



Membrane was stained with AzureRed (gray) and probed for three different proteins using AzureSpectra secondary antibodies (AzureSpectra 490 – blue, AzureSpectra 700 – red, AzureSpectra 800 – green). The resulting fluorescent Western blot was scanned on a Sapphire Biomolecular Imager to detect all three proteins and the total protein stain simultaneously.

Detailed Protocol

Step 1. Washing

- Following transfer, wash blot for 5 min in water.
- Proceed to PVDF (2) or Nitrocellulose (3) protocol.

Notes

- For best results, run the buffer front off the base of the gel during electrophoresis prior to transfer.
- Do not allow membrane to dry during staining.
- For all steps, use 50 ml for small blots, 400 ml for large.

Step 2. PVDF Protocol

2.a. Staining

- Prepare Stain Solution: Allow AzureRed Dye to warm to room temperature. Mix thoroughly. For small blots, dilute 125 µl AzureRed Dye in 50 ml Stain Buffer. Mix well.
- For large blots, dilute 1 ml of AzureRed Dye in 400 ml Stain Buffer. Mix well.
- Place blot protein side down in Stain Solution.
- Stain blot with gentle rocking for 15–30 min.

2.b. Acidification

- Place blot in Fix Solution and incubate with gentle rocking for 5 min.

Notes

- Blot may appear green.

2.c. Wash

- Rinse blot 3 times with 100% ethanol for 2–3 min each, until green background on blot has been completely removed.

Notes

- Methanol may be used instead of ethanol.

2.c. Drying

- Hang blot from a peg or dry on wire mesh to allow blot to dry evenly.
- Allow blot to dry completely before imaging.

Notes

- If using in a multiplex Western blot, do not dry the membrane before blotting. Proceed to the blocking step of your Western blot protocol immediately after washing.

Step 3. Nitrocellulose Protocol

3.a. Staining

- Prepare Stain Solution: Allow AzureRed Dye to warm to room temperature. Mix thoroughly. For small blots, dilute 125 µl AzureRed Dye in 50 ml Stain Buffer. Mix well.
- For large blots, dilute 1 ml of AzureRed Dye in 400 ml Stain Buffer. Mix well.
- Place blot protein side down in Stain Solution.
- Stain blot with gentle rocking for 15–30 min.

3.b. Acidification

- Place blot in Fix Solution and incubate with gentle rocking for 5 min.

Notes

- Blot may appear green.

3.c. Washing

- Wash blot 1 time in Wash Solution for 5 min with gentle rocking.
- Wash blot 2 times in highpurity water for 5 min with gentle rocking.

3.d. Drying

- Allow blot to dry completely before imaging.

Notes

- If using in a multiplex Western blot, do not dry the membrane before blotting. Proceed to the blocking step of your Western blot protocol immediately after washing.

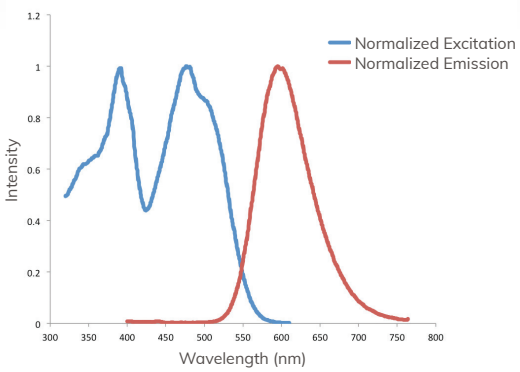


Figure 1. Excitation and Emission Spectra of AzureRed Dye.

Azure Imager	Recommended Imaging Channel
c150, c200, c280, c300, c500	UV365
c400, c600	Cy3
Sapphire Biomolecular Imager – RGB, RGBNIR	520

Table 1. AzureRed is imageable with both UV and green light. However, best sensitivity is achieved when detected with green light.