

PVDF Staining Protocol continued

3. Staining: Place blot in Working Stain and stain with gentle rocking for 15–30 min.
4. Acidification: Place blot in fix solution and incubate with gentle rocking for 5 min. Blot may appear green.
5. Wash: Rinse blot 3 times for 2-3 min each wash in 100% ethanol or HPLC grade methanol.
6. For use in multiplex Western blotting, proceed directly to blocking step after wash. Otherwise, blot may be dried after washing.

Imaging

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

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

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AzureRed Fluorescent Protein Stain

Fluorescent total protein stain for gels and blots

Short Protocol for Catalog Number

AC2124 AzureRed Fluorescent Protein Stain, 5 ml

DC0019-002



Description

AzureRed Fluorescent Protein Stain provides sub-nanogram sensitivity with a quick, 30 minute protocol. This total protein stain is quantitative, nontoxic, and fully compatible with downstream applications including Western blotting and mass spectrometry.

Features

- **Sensitive** – detect less than a nanogram of protein per spot or band
- **Convenient** – simple 30 minute membrane protocol
- **Flexible** – stain gels or membranes
- **Reversible** – destain if desired for downstream analysis
- **No speckling** – clean background and no speckling for better data
- **Environmentally friendly** – biodegradable and contains no heavy metals for increased safety and simple disposal
- **Compatible** – image with UV or green light. Multiplex stained membranes with fluorescent Western blots for simultaneous detection of total protein and proteins of interest.

Quick Protocol

Preparation of Solutions

The amount of reagents in each packet of AzureRed Powder A or B is sufficient to prepare 1 L of solution. To prepare larger volumes, use more than one packet.

1. **Fix Solution:** Add the contents of one AzureRed Powder A packet (10.1 g) to 850 ml of high-purity water (distilled, Milli-Q®, or equivalent) in a 1 L bottle. Add 150 ml 100% ethanol and mix thoroughly.
2. **Stain Buffer:** Add the contents of one AzureRed Powder B packet (23.4 g) to 1 L of high-purity water in a 1 L bottle. Dissolve completely.
3. **Wash Solution:** Mix 850 ml high-purity water and 150 ml 100% ethanol in a 1 L bottle.

PVDF Membrane Stain Protocol

For correct volumes at each step, refer to Table 2 in the AzureRed Fluorescent Protein Stain Long Protocol found on www.azurebiosystems.com.

1. **Washing:** Following transfer, wash blot in ultrapure water for 5 min.
2. **Prepare Working Stain:** For a 9x7cm membrane, dilute 125uL AzureRed Dye in 50mL Stain Solution.