AzureRed Fluorescent Protein Stain

Fluorescent total protein stain for gels and blots

Protocol for Catalog Number

AC2124 AzureRed Fluorescent Protein Stain

www.azurebiosystems.com • info@azurebiosystems.com

DC0021-003

azure biosystems

Important Information

The following instructions are for use with AzureRed total protein stain, catalog number AC2124. Please see the Kit Contents section for details.

Storage Information

Store AzureRed Dye in a freezer at -15 ºC to -30 ºC in the original brown bottle provided and protect from light. The AzureRed Powder A and Powder B are stable at room temperature for one year.

Warnings and Precautions

- AzureRed total protein stain is for research use only.
- Always wear gloves when handling membranes and reagents.
- Refer to MSDS for additional safety information.
- The product is guaranteed to be free of manufacturer defect, and to function as described when the enclosed protocol is followed by properly trained personnel. Please see the Warranty section for more information.

Table of Contents

1. Kit Contents

AC2124:

AzureRed total protein stain, 5 ml

• AzureRed Powder A	4 packets, 10.1 g each
• AzureRed Powder B	23.4 g
• AzureRed Dye	5 ml

AzureRed Stain, 5 ml, kit is sufficient for staining twenty SDS-PAGE mini-gels (8 cm x 11 cm), four fill-sized 2D gels (17 cm x 17 cm), or forty mini-blots (9 cm x 7 cm).

2. Shipping and Storage Conditions

Product may be shipped refrigerated or frozen on blue ice or dry ice. Shipping at ambient temperature (below 27°C) is acceptable if the total dispatch time is no longer than 5 days. Upon receipt, store AzureRed Dye in a freezer at -15 °C to -30 °C in the original brown bottle provided and protect from light. AzureRed Powder A and Powder B may be stored at room temperature in a dry location.

3. Additional Materials Required

- High-purity water (distilled, Milli Q, or equivalent)
- 100% ethanol
- Staining tray
- Shaking or rocking platform

4. About AzureRed

AzureRed is based on epicocconone, a small, naturally occurring fluorescent compound[1] that reversibly binds to lysine, arginine, and histidine residues in proteins and peptides to yield an intensely red-fluorescent product.[2] This unique mechanism provides sensitive quantification of proteins in 1D and 2D gels of all chemistries, on both PVDF and nitrocellulose blots[3-5] and provides unparalleled compatibility with Mass Spectrometry.[6-8]

5. Excitation and Emission Spectra

The excitation and emission spectra of AzureRed can be seen in Figure 1.

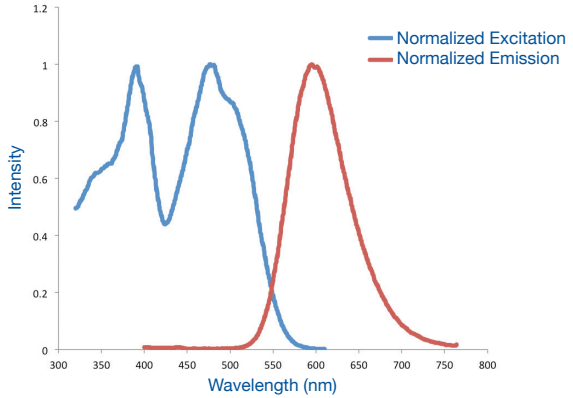

Figure 1. Excitation and Emission Spectra of AzureRed Dye

6. Overview of AzureRed Membrane Staining Protocol

Rinse
Rinse membrane in ultra pure water for 5 minutes

↓

Staining
Incubate membrane in Stain Solution for 15 to 30 minutes

↓

Fixation
Incubate membrane in Fix Solution for 5 minutes

↓

Washing
Wash membrane in methanol 2 times for 3 minutes each

↓

Image or Proceed with Western Blotting

7. 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.

Gel Dimensions	Solution				
	Fix	Stain		Wash	Fix
		Stain Buffer	AzureRed Dye		
8 cm x 11 cm x 1 mm (mini-gels)	100 ml	50 ml	250 µL	100 ml	100 ml
13.3 cm x 8.7 cm x 1 mm (small format 2D gels)	200 ml	100 ml	500 µL	200 ml	200 ml
17 cm x 17 cm x 1 mm	500 ml	250 ml	1.25 ml	500 ml	500 ml
17 cm x 17 cm x 1.5 mm	500 ml	250 ml	1.25 ml	500 ml	500 ml
15 cm x 19 cm x 1 mm	500 ml	250 ml	1.25 ml	500 ml	500 ml
15 cm x 19 cm x 1.5 mm	500 ml	250 ml	1.25 ml	500 ml	500 ml
20 cm x 25 cm x 1 mm	750 ml	375 ml	1.875 ml	750 ml	750 ml
20 cm x 25 cm x 1.5 mm	750 ml	375 ml	1.875 ml	750 ml	750 ml

Table 2. Volumes of Solutions For Different Gel Sizes

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

Table 3. AzureRed is imageable with both UV and green light. However, best sensitivity is achieved when detected with green light.

8. Detailed Protocol, Blot Staining

Step	Notes
1. Washing	
• Following transfer, wash blot for 5 min in water. • Proceed to PVDF (2) or Nitrocellulose (3) protocol.	• 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.
2. PVDF Protocol	
2a. Staining • Place blot protein side down in Stain Solution. • Stain blot with gentle rocking for 15–30 min.	• 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.
2b. Acidification • Place blot in Fix Solution and incubate with gentle rocking for 5 min.	• Blot will appear green.
2c. Wash • Rinse blot 3 times with 100% ethanol for 2–3 min each, until green background on blot has been completely removed.	• Methanol may used instead of ethanol.
2d. Drying • Hang blot from a peg or dry on wire mesh to allow blot to dry evenly. • Allow blot to dry completely before imaging.	• If using in a multiplex Western blot, do not dry membrane after staining. Upon completion of the washing step, proceed directly to blocking the membrane.

8. Detailed Protocol, Blot Staining, continued

Step	Notes
3. Nitrocellulose Protocol	
3a. Staining • Place blot protein side down in Stain Solution. • Stain blot with gentle rocking for 15–30 min.	• 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.
3b. Acidification • Place blot in Fix Solution and incubate with gentle rocking for 5 min.	• Blot will appear green.
3c. Washing • Wash blot 1 time in Wash Solution for 5 min with gentle rocking. • Wash blot 2 times in high-purity water for 5 min with gentle rocking.	
3d. Drying • Allow blot to dry completely before imaging.	• If using in a multiplex Western blot, do not dry membrane after staining. Upon completion of the washing step, proceed directly to blocking the membrane.

9. Detailed Protocol, Gel Staining

Step	Notes
1. Fixation	
• Fix gel in Fix Solution for a minimum of 1 hr with gentle rocking. • For correct volumes at each step, refer to Table 2.	• For gels thicker than 1 mm or backed gels, increase the fixation time to 1.5 hr. • The fixation time can be extended to overnight.
2. Staining	
• Prepare the Stain Solution immediately prior to staining. • Remove gel from Fix Solution and place in Stain Solution, minimizing carryover of the fixing solution. • Stain gel for 1 hour with gentle rocking.	• To prepare Stain Solution: Allow AzureRed Dye to warm to room temperature. Mix thoroughly, then dilute 1 part AzureRed Dye in 200 parts Stain Buffer. Mix well. Refer to Table 2 for volumes of solutions used for different gel sizes. • Stain Solution will degrade over time. Prepare only as much as is needed and use immediately. • Increase staining time to 1.5 hours for gels 1.5 mm thick or backed gels. Extending the staining time to 2 hours will not affect results. • DO NOT stain for longer than 2 hours.
3. Washing	
• Remove gel from Stain Solution, rinse with high-purity water, and wash in Wash Solution for 30 min with gentle rocking.	• For 1.5 mm thick gels, or gels with high background fluorescence, increase washing time to 45 min.

9. Detailed Protocol, Gel Staining, continued

Step	Notes
4. Acidification	
• Remove gel from Wash Solution and place in Fix Solution. • Incubate in Fix Solution for 30 min with gentle rocking.	• This step can be repeated or extended to overnight to reduce background staining. • If performing this step overnight, protect the gel from light.
5. Imaging	
• Detect fluorescence at 610 nm using standard fluorescence scanners and CCD camera systems. For recommended imaging settings, refer to Table 3.	• Compatible excitation sources include green light and UV light. • Detect fluorescence using a 610 nm band pass or 560 nm long pass filter.

10. Destaining

AzureRed staining is reversible and the stain may be removed for subsequent analysis such as Western blotting.

1. **To destain while minimizing protein loss:**
   Wash blot overnight in 50 mM ammonium carbonate.
2. **To rapidly destain PVDF membranes:**
   Wash blot with 50% acetonitrile containing 30 mM ammonium carbonate for 15 min.
3. **To rapidly destain nitrocellulose membranes:**
   Wash blot with 50% ethanol or methanol containing 50 mM ammonium carbonate for 15 min.
4. **To rapidly destain protein gels:**
   Wash blot with 50% ethanol or methanol containing 50 mM ammonium carbonate for 15 min to 1 hour.

11. Storage

Gels may be stored at 4 ºC in 1% citric acid and protected from light. For extended storage (up to 6 months), add AzureRed Dye to the storage solution at 1:200. Prior to imaging, rinse gels 2 x 15 min in Wash Solution. Incubating in Fix Solution for 15 minutes can reduce background.

Blots may be stored dry, in the dark, at room temperature.

12. Troubleshooting & FAQ

Problem	Possible Soutions
High background	• Handle gels with clean non-powdered gloves, and avoid contamination with dust. • Ensure concentrated AzureRed Dye was brought to room temperature and thoroughly mixed prior to dilution. • Ensure stain was thoroughly mixed into Stain Buffer before adding to gel. • Stain only one gel per tray. • Use high-purity water (distilled, Milli-Q, or equivalent).
No or low signal	• Check pH during staining step; pH should be between 9.5 and 10.5. Carry-over acid from the fixation step can result in poor staining. • Stain may fade with long exposure times and associated heating on CCD-based instruments. • Ensure stain concentrate was brought to room temperature and mixed thoroughly before dilution. • Staining for over 2 hours in alkaline conditions destabilizes proteins, and leads to diffusion of protein bands from the gel matrix.
Negative staining	• Use high-quality SDS in the preparation and running of the gel. • Extend fixation time to overnight. • Use correct volumes of Fix and Wash Solutions. • Extend washing time.
Speckled background	• Filter buffers to remove dust or precipitates. • Protect gel from airborne particles.

13. References

1. Bell, P.J.L. and Karuso, P. (2003). Epicocconone, a novel fluorescent compound from the fungus Epicoccum nigrum. *Journal of the American Chemical Society.* 125, 9304-9305.
2. Coghlan, D. R., Mackintosh, J. & Karuso, P. (2005). Mechanism of reversible fluorescent staining of protein with Epicocconone. *Organic Letters.* 7, 2401-240.
3. Mackintosh, J.A., Veal, D.A. and Karuso, P. (2005). FluoroProfile, a fluorescence based assay for rapid and sensitive quantification of proteins in solution. *Proteomics.* 5, 4673-4677.
4. Malmport, E., Mackintosh, J., Ji, H., Veal, D. & Karuso, P. (2005). Visualization of proteins electro-transferred on Hybond ECL and Hybond-P using Deep Purple Total Protein Stain. *GE-Healthcare Life Science News.* 19, 12-13.
5. Mackintosh, J.A., Choi, H.-Y., Bae, S.-H., Veal, D.A., Bell, P.J., Ferrari, B.C., van Dyk, D., Verrills, N.M., Paik, Y.-K. & Karuso, P. (2003). A fluorescent natural product for ultra sensitive detection of proteins in 1-D and 2-D gel electrophoresis. *Proteomics.* 3, 2273-2288.
6. Tannu, N.S. Sanchez Brambila, G.S., Kirby, P., Andacht, T.M. (2006). Effect of staining reagent on peptide mass fingerprinting from in-gel trypsin digestions: A comparison of Sypro Ruby and Deep Purple. *Electrophoresis.* 27, 3136 - 3143.
7. Nock, C.M., Ball, M. S., White, I. R., Skehel, J. M., Bill, L. and Karuso P. (2008). Mass Spectrometric Compatibility of Deep Purple and SYPRO Ruby total protein stains for high throughput proteomics using large format two dimensional gel electrophoresis. *Rapid Communications in Mass Spectrometry.* 22, 881-886.
8. Ball, M. S., Karuso, P. (2007). Mass Spectral Compatibility of Four Proteomics Stains. *Journal of Proteome Research.* 6, 4313-4320.

14. Related Products

Catalog Number	Product	Size
AC2105	Low Fluorescence Western Membrane (PVDF) 7x9 cm	10 sheets
AC2106	Nitrocellulose Transfer Membrane 0.45 µm 7x9 cm	10 sheets
AC2107	Nitrocellulose Transfer Membrane 0.22 µm 7x9 cm	10 sheets

15. Warranty

This product is warranted to be free of defects of material or workmanship, and to perform as described in the published specifications when stored according to the documentation included with the product, and used according to the accompanying instruction manual by appropriately trained personnel. If the product is found to have a defect upon first use and within 30 days of shipment, the product may be replaced. This warranty extends only to the original purchaser of the product. There is no obligation to replace the product as a result of misuse, improper storage, or negligence of the buyer.

16. User Notes