User Manual

AdvanStain™ Scarlet™

Fluorescent total protein stain for gels and blots

For Catalog Numbers

K-11072-B50 AdvanStain Scarlet Kit, 5 ml, dilutes to 1 L

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Important Information

The following instructions are for use with AdvanStain Scarlet total protein stain, catalog numbers K-11072-250 and K-11072-B50. Please see the Kit Contents section for details.

Storage Information

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

Warnings and Precautions

- AdvanStain Scarlet total protein stain is for research use only.
- The AdvanStain Scarlet total protein stain is compatible with PVDF membranes.
- Always wear gloves when handling membranes and reagents.
- Refer to SDS 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.

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1. Kit Contents

Catalog Number: K-11072-B50, AdvanStain Scarlet total protein stain, 5 ml

- R-04016-C10, AdvanStain Scarlet Powder A, 4 x 10.1 g
- R-04017-C23, AdvanStain Scarlet Powder B, 23.4 g
- R-03016-B50, AdvanStain Scarlet Dye, 5 ml

AdvanStain Scarlet Stain, 5 ml, kit is sufficient for staining twenty SDS-PAGE mini-gels (8 cm x 11 cm) or four full-size 2-D gels

2. Shipping and Storage Conditions

Product is shipped at room temperature. Upon receipt, store AdvanStain Scarlet Dye in a freezer at -15 °C to -30 °C in the original brown bottle provided and protect from light.

AdvanStain Scarlet Powder A and Powder B may be stored at room temperature in a dry location. (17 cm x 17 cm).

3. Additional Materials Required

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

4. About AdvanStain Scarlet

AdvanStain Scarlet 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 and PVDF blots and provides unparalleled compatibility with Mass Spectrometry.[6-8]

5. Excitation and Emission Spectra

Optimal excitation wavelengths for AdvanStain Scarlet are 405 or 500 nm. Compatible excitation light sources include green (543, 532 nm), blue (488 nm), violet (405 nm) or UV (302/365 nm).

The maximum emission wavelength of AdvanStain Scarlet stain is 610 nm regardless of what excitation source is used. 610 nm band pass or 560 long pass filters may be used. The excitation and emission spectra of AdvanStain Scarlet can be seen in Figure 1.

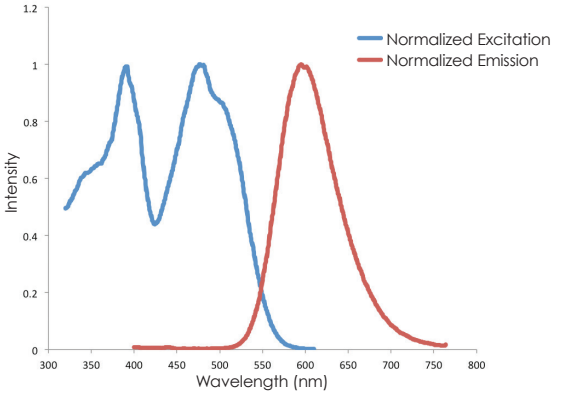
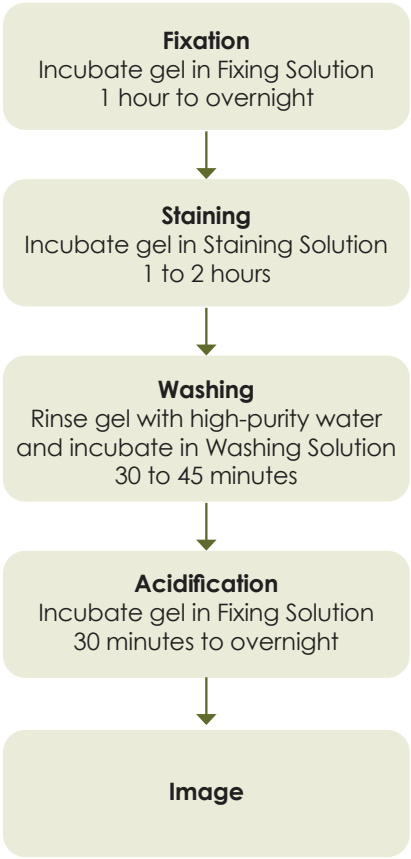


Figure 1. Excitation and Emission Spectra of AdvanStain Scarlet Dye

6. Overview of AdvanStain Scarlet Gel Staining Protocol



7. Preparation of Solutions

Before staining, prepare Fixing, Staining and Washing 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 container of AdvanStain Fixing Solution or Staining Solution is sufficient to prepare 1 L of solution. Do not split the containers. Once a container is opened, the entire contents should be used. For preparation of larger volumes, use more than one pouch.

Fixing Solution
Add contents of one AdvanStain Scarlet Powder A container (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 AdvanStain Scarlet Powder B container (23.4 g) to 1 L of high-purity water in a 1 L bottle. Mix until completely dissolved.

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

8. Detailed Protocol, Gel Staining

Step	Notes
1. Fixation • Fix gel in Fixing 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 Staining Solution immediately prior to staining. • Remove gel from Fixing Solution and place in Staining Solution, minimizing carry-over of the fixing solution. • Stain gel for 1 hour with gentle rocking.	• To prepare Staining Solution: Allow AdvanStain Scarlet Dye to warm to room temperature. Mix thoroughly, then dilute 1 part AdvanStain Scarlet Dye in 200 parts Staining Buffer. Mix well. Refer to Table 2 for volumes of solutions used for different gel sizes. • Staining 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 Staining Solution, rinse with high-purity water, and wash in Washing 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.
4. Acidification • Remove gel from Washing Solution and place in Fixing Solution. • Incubate in Fixing 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 (543, 532 nm), blue (488 nm), violet (405 nm), or UV (302/365 nm). • Detect fluorescence using a 610 nm band pass or 560 nm long pass filter.

Gel Dimensions	Solution				
	Fixing	Staining		Washing	Fixing
		Stain Buffer	AdvanStain Scarlet 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

Imaging System	Excitation	Emission	Notes
Laser scanner	Green (532 nm) light	Orange long pass (560 nm) filter or red (610 nm) filter	
CCD imager with transilluminator	Long wavelength UV (302/365 nm) or black light blue lamp	Orange long pass filter	
Ettan™ DIGE Imager (GE Healthcare)	Green (540/25 nm) light source	Orange (595/25 nm) filter	For multiplex applications, violet excitation filter (390/20 nm) and orange emission filter will avoid cross talk with Cy2 and Cy3

Table 3. Recommended Imaging Conditions for Different Imaging Systems

9. Detailed Protocol, Blot Staining

Step	Notes
1. Washing • Following transfer, wash blot for 5 min in water.	• 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. Staining • Place blot protein side down in Staining Solution. • Stain blot with gentle rocking for 15–30 min.	• Prepare Staining Solution: Allow AdvanStain Scarlet Dye to warm to room temperature. Mix thoroughly. For small blots, dilute 125 µl AdvanStain Scarlet Dye in 50 ml Stain Buffer. Mix well. • For large blots, dilute 1 ml of AdvanStain Scarlet Dye in 400 ml Stain Buffer. Mix well.
3. Acidification • Place blot in Fixing Solution and incubate with gentle rocking for 5 min.	Blot will appear pink.
4. 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.
5. Drying • Hang blot from a peg or dry on wire mesh to allow blot to dry evenly. • Allow blot to dry completely before imaging.	

10. *Destaining*

AdvanStain Scarlet 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 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 °C in 1% citric acid and protected from light. For extended storage (up to 6 months), add AdvanStain Scarlet Dye to the storage solution at 1:200. Prior to imaging, rinse gels 2 x 15 min in Washing Solution. Incubating in Fixing Solution for 15 minutes can reduce background.

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

12. Troubleshooting & FAQ

Problem	Possible Solutions
High background	• Handle gels with clean non-powdered gloves, and avoid contamination with dust. • Ensure concentrated AdvanStain Scarlet Dye was brought to room temperature and thoroughly mixed prior to dilution. • Ensure stain was thoroughly mixed into Staining 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 Fixing and Washing Solutions. • Extend washing time.
Speckled background	• Filter buffers to remove dust or precipitates. • Protect gel from airborne particles.

13. References

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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
K-02101-010	Afyon™ SDS-PAGE Sample Preparation Kit	10 rxns
K-02101-025	Afyon™ SDS-PAGE Sample Preparation Kit	25 rxns
K-12045-C20	WesternBright® ECL Western Blotting HRP Substrate trial kit size	20 ml
K-12045-D20	WesternBright® ECL Western Blotting HRP Substrate (for 2000 cm^2 membrane)	200 ml
K-12045-D50	WesternBright® ECL Western Blotting HRP Substrate (for 5000 cm^2 membrane)	500 ml
L-08001-010	Low Fluorescence Western Membrane (PVDF) 7x9 cm	10 sheets
L-08002-010	Nitrocellulose Transfer Membrane 0.45 µm 7x9 cm	10 sheets
L-08003-010	Nitrocellulose Transfer Membrane 0.22 µm 7x9 cm	10 sheets
R-03018-B10	Non-reducing protein sample loading buffer (2X)	1 ml
R-03018-B50	Non-reducing protein sample loading buffer (2X)	5 ml
R-03019-B10	Reducing protein sample loading buffer (2X)	1 ml
R-03019-B50	Reducing protein sample loading buffer (2X)	5 ml

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

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