

## Troubleshooting & FAQ, continued

Problem	Possible Solutions
No or low signal	<ul style="list-style-type: none"><li>• Ensure effective electrophoretic transfer: check electrical source and verify transfer was performed in the right direction, optimize the transfer time and voltage conditions, ensure good contact was established between membrane and gel, make sure proper transfer buffer was used.</li><li>• If using PVDF, check that the membrane is fully hydrated before incubating with stain. Rewet in methanol if necessary.</li></ul>
Uneven staining	<ul style="list-style-type: none"><li>• Check that the blot is completely immersed in the staining solution.</li><li>• Ensure even agitation by incubating on orbital shaker.</li><li>• Improve transfer, making sure that the membrane and the gel are assembled correctly and tightly in your transfer apparatus, and adequate pressure is applied.</li></ul>

## For More Information



visit <http://advansta.com/products/AdvanStain-Iris> or go directly to the web-page by scanning the QR-code with your favorite bar-code scanner app on your smart phone.

### Advansta Corporation

2140 Bering Drive | San Jose, CA 95131

Tel: 650.325.1980 | Fax: 650.325.1904

Email: [support@advansta.com](mailto:support@advansta.com)

[www.advantsta.com](http://www.advantsta.com)

## AdvanStain™ Iris

High sensitivity stain for nitrocellulose  
and PVDF membranes

## For Catalog Number

**R-03732-D25** AdvanStain Iris, 250mL



### Storage Information

AdvanStain Iris is stable at room temperature (4°-25°C) for at least one year.

### Warnings and Precautions

- AdvanStain Iris is for research use only.
- Always wear gloves when handling membranes and reagents.
- Refer to SDS for additional safety information.
- The product is guaranteed to be free of manufacturer defects, and to function as described when the provided protocol is followed by properly trained personnel.

### Short Protocol

**Note:** This protocol is for staining one full-size mini-blot. If using a larger blot, adjust the volume of staining and washing solution appropriately.

1. Following the transfer, immerse the blot in at least 10mL of water for 5 minutes with gentle agitation.
2. Decant water and dispense 10mL of AdvanStain Iris solution to the mini-blot, ensuring that it is completely immersed for 10 minutes with gentle agitation.

3. Rinse the blot with high quality water.
  - 3X quickly with ~25mL water for ~5 seconds per rinse
4. Image the blot for permanent record. If imaging wet, immediately place the blot in a Imaging Folder before imaging. If imaging dry, allow the blot to completely dry (no visible streaks should be observed).
5. Following imaging, immerse the blot in at least 10mL of water for 5 minutes with gentle agitation before proceeding with Western blotting. If the PVDF blot was imaged dry, rewet with methanol prior to rinsing with water.

### Troubleshooting & FAQ

AdvanStain Iris is a quick and easy method for staining membrane-bound proteins and to check the quality of protein transfer before performing a Western blot assay. Some common questions are addressed below.

Problem	Possible Solutions
High background	<ul style="list-style-type: none"><li>• Increase washing time with water.</li></ul>
White spots within bands	<ul style="list-style-type: none"><li>• Improve transfer, making sure to remove any bubbles between the gel and the membrane.</li></ul>