Troubleshooting & FAQ

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

Problem	Possible Solutions
High background	• Increase washing time with water.
No or low signal	• 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. • If using PVDF, check that the membrane is fully hydrated before incubating with stain. Rewet in methanol if necessary.
White spots within bands	• Improve transfer, making sure to remove any bubbles between the gel and the membrane.
Uneven staining	• Check that the blot is completely immersed in the staining solution. • Ensure even agitation by incubating on orbital shaker. • Improve transfer, making sure that the membrane and the gel are assembled correctly and tightly in your transfer apparatus, and adequate pressure is applied.

For detailed troubleshooting guide for protein analysis, please visit our website at www.advansta.com/Troubleshooting

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AdvanStain™ Ponceau

Quick protein stain for membranes

For Catalog Numbers

R-03021-D50 AdvanStain Ponceau, 500 ml

advansta

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Storage Information

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

Warnings and Precautions:

- AdvanStain Ponceau 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 defects, and to function as described when the provided protocol is followed by properly trained personnel.

For More Information

including a detailed manual, visit **www.advansta.com/AdvanStain_Ponceau** or go directly to the web-page by scanning the QR-code with your favorite bar-code scanner app on your smart phone.

Short Protocol

Step
1. Following the transfer, wash your blot in copious amount of high purity water for 10 minutes.
2. Immerse the blot in 10-20 ml of AdvanStain Ponceau solution (at least 0.2 ml/cm^2 membrane) and stain for 5 minutes on orbital shaker.
3. Drain off and decant the staining solution and rinse the membrane with high purity water until the background is reduced. Avoid prolonged rinsing which may destain the bands.
4. Image the blot for permanent record.
5. Destain the blot in water for 10-20 minutes. Complete destaining is achieved during subsequent blocking step during which remaining AdvanStain Ponceau dye will not interfere with blocking or antibody binding.
6. Continue with Western blot procedure.