Troubleshooting & FAQ

AdvanStain Total-PVDF Fluorescent Protein Staining Kit 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 Cause
High background	• Increase washing time.
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. • Ensure that the PVDF membrane is fully hydrated before incubating with stain. Rewet in methanol if necessary, followed by water.
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 More Information

visit **www.advansta.com/products/AdvanStain-Total** or go directly to the web-page by scanning the QR-code with your favorite bar-code scanner app on your smart phone.

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AdvanStain™ Total-PVDF Fluorescent Protein Staining Kit

Rapid fluorescent stain for total protein normalization

For Catalog Number

K-11061-015 AdvanStain Total-PVDF Fluorescent Protein Staining, 15 mini-blots

Description

AdvanStain Total is a fluorescent protein stain for PVDF membranes that allows for sensitive and quantitative visualization of protein bands. For precise quantitative Western blot data, it is critical to perform normalization to account for sample inconsistencies, sample loading errors and uneven transfer. In an effort to increase transparency, total protein normalization is quickly becoming the standard for Western blot normalization as it is now the preferred method for top tier journals. The AdvanStain Total-PVDF Fluorescent Protein Staining Kit provides a convenient method for total protein normalization that is compatible with digital imaging systems equipped with a Cy3 filter.

advansta December 16, 2020 | D-26044-413

Kit Contents

- R-03143-D15, PVDF Staining Solution
- R-03144-D45, Washing Solution
- L-07020-015, Imaging Folders

Additional Materials Required

- 100% Ethanol (use only pure, 200-proof ethanol)
- Staining tray
- Shaking or rocking platform

Storage Information

The AdvanStain Total-PVDF Fluorescent Protein Staining Kit is stable at room temperature (4°–25°C) for at least one year.

Warnings and Precautions

- The AdvanStain Total-PVDF Fluorescent Protein Staining Kit is for research use only.
- The AdvanStain Total-PVDF Fluorescent Protein Staining Kit is compatible with PVDF membranes.
- If staining nitrocellulose membranes use our alternative product K-11062-015.
- 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.

Preparation of Solutions

- **Washing Solution:** Before using the product for the first time, add 135ml of Ethanol directly to the bottle of Washing Solution and mix well. Check the box on the bottle's label. This only needs to be done one time.

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 5–10mL of PVDF Staining Solution for 5 minutes with gentle agitation.
3. Decant the PVDF Staining Solution and wash the membrane three times for 3 minutes per wash with 5–10mL Washing Solution.
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 blot was imaged dry, rewet with methanol prior to rinsing with water.