Tips

- Western blotting requires optimization of primary and secondary antibody concentrations used in steps 3 and 5 of the Short Protocol. These must be determined empirically for every antigen-antibody pair.
- Fluorescent Western blotting can be performed as a common procedure utilizing a primary antibody and a fluorescently labeled secondary antibody. Alternatively, directly labeled primary antibodies may be used, which eliminates the need of secondary antibody and shortens the procedure. Adjust the protocol appropriately if using directly labeled primary antibodies.
- When imaging a dry blot, ensure that the blot is completely dry. Semi-dry blots will have visible streaks caused by non-uniform wetness. Imaging a blot that is not uniformly wet or dry will cause data inconsistencies.
- Do not touch the membrane with fingers or dirty forceps as this may result in non-specific background.

For More Information

visit **products.advansta.com/LightSaver-Fluorescence-Enhancing-Solution** 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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LightSaver™ Fluorescence Enhancing Solution

Enhance fluorescent Western blot signal

For Catalog Number

R-03105-D10	LightSaver™ Fluorescence Enhancing Solution, 100mL

advansta

August 27, 2024 | D-26045-403

LightSaver™ Fluorescence Enhancing Solution

Description

LightSaver™ Fluorescence Enhancing Solution is a ready-to-use buffer for fluorescent Western blotting. It is designed to enhance fluorescent signal on dried blots and is compatible with PVDF and Nitrocellulose membranes. This buffer provides ultimate flexibility as it may be applied after preliminary images of a wet blot are acquired. After rinsing with LightSaver™, allow the blot to dry, then image for enhanced sensitivity.

Storage Information

The LightSaver™ Fluorescence Enhancing Solution is stable at room temperature (4°–25°C) for at least one year.

Warnings and Precautions

- The LightSaver™ Fluorescence Enhancing Solution is for research use only.
- The LightSaver™ Fluorescence Enhancing Solution is compatible with PVDF and Nitrocellulose 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 defects, and to function as described when the provided protocol is followed by properly trained personnel.

Short Protocol

1. Prepare your protein blot on either a PVDF of Nitrocellulose membrane using your standard technique.
2. Block the membrane for one hour at ambient temperature with gentle agitation using a sufficient volume of buffer to completely cover the membrane.
3. Incubate with the primary antibody diluted in blocking buffer for one hour at ambient temperature with gentle agitation.
4. Wash the blot with AdvanWash™ Washing Solution, PBST or TBST:
   - 2 x quickly (~5 seconds per rinse)
   - 3 x 5 minutes, with at least 0.3mL/cm^2 membrane each time
5. Incubate with the secondary antibody diluted in blocking buffer for one hour at ambient temperature with gentle agitation.
6. Wash the blot with AdvanWash™ or AdvanWash™-IR Washing Solution, PBST or TBST:
   - 2 x quickly (~5 seconds per rinse)
   - 3 x 5 minutes, with at least 0.3mL/cm^2 membrane each time
7. Rinse the blot for 5 minutes with 1X PBS to remove detergent which may cause elevated fluorescent background.
8. Rinse the blot for 5 minutes at ambient temperature with gentle agitation using a sufficient volume of LightSaver™ Fluorescence Enhancing Solution to completely cover the membrane.
9. For best results, image the blot dry.