Troubleshooting and FAQ

Western blots may be difficult to troubleshoot due to the multiple steps that are involved. Some common problems that are encountered are addressed below:

Problem	Possible Solutions
Inconsistent transfer caused by air bubbles or poor contact between the gel and the membrane Uneven contact between the gel and the membrane	• Use a roller to remove any air bubbles that may have formed between the gel and the membrane.
Too much heat generated during the transfer	• Ensure that the semi-dry blotting paper is completely saturated prior to transfer. • If high heat production is persistent, shorten the duration of transfer, decrease the current, or increase the thickness of each blotting paper stack.
Blotting paper dried out during transfer	• Ensure that the semi-dry blotting paper is completely saturated prior to transfer. • Complete assembly of the semi-dry blotting sandwich within 15 minutes.
No transfer of proteins	• Use pre-stained molecular weight markers to monitor the transfer
Poor or incomplete transfer of proteins	• Ensure that the gel, membrane and blotting paper are all cut to the same size, with no overhanging material. If they are not, it is possible for the current to bypass the transfer stack. • Coomassie stain the post-transfer gel to check for residual proteins

For More Information

including a more detailed protocol, visit **www.advansta.com/products/FLASHBlot-SD-semi-dry-transfer-buffer** 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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FLASHBlot™-SD Transfer Buffer

Rapid high efficiency semi-dry transfer buffer

For Catalog Number

R-03104-D50 FLASHBlot™-SD Transfer Buffer, 500mL

Description

FLASHBlot™-SD Transfer Buffer is designed for rapid semi-dry transfer of proteins from polyacrylamide gels (SDS-PAGE) to nitrocellulose or PVDF membranes using rapid semi-dry transfer systems. Transfer is compatible with commonly used detection methods such as membrane staining, chemiluminescent and fluorescent Western blotting.

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Additional Materials Required

- Semi-Dry Transfer Apparatus
- Semi-Dry Blotting Tissue or Paper
- PVDF or Nitrocellulose Membrane

Storage Information

FLASHBlot-SD is stable for at least 12 months when stored properly at room temperature.

Warnings and Precautions

- FLASHBlot-SD is for research use only.
- Always wear gloves when handling reagents.
- Refer to MSDS for additional safety information.
- This product is guaranteed to be free of manufacturer defects, and to function as described when the enclosed protocol is followed by properly trained personnel.

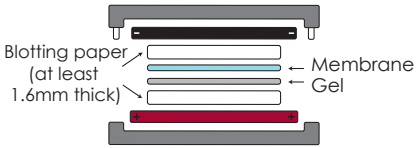
Tips

- Cut the blotting tissue or filter paper and membrane to fit the gel, ensuring that the gel does not touch the anode surface.
- Never touch the membrane or gel with bare hands. Use forceps to make adjustments.
- Use semi-dry blotting tissue or paper, standard cellulose blotting paper or thick filter paper may overheat during transfer.
- Clean blotting apparatus with water after every use.

Short Protocol

Note: FLASHBlot-SD is supplied as a ready-to-use 1X solution for semi-dry Western blotting.

1. Equilibriate two stacks of semi-dry blotting tissue or paper, each equivalent to at least 1.6mm for 10–15 minutes. Ensure that the blotting paper is completely soaked prior to use.
2. Equilibriate the Nitrocellulose or PVDF membrane in FLASHBlot-SD transfer buffer for 10–15 minutes, use sufficient buffer to cover the entire membrane. *(For PVDF membranes, pre-wet with methanol for 10–15 seconds then rinse with high purity water for 5 minutes before equilibriating with FLASHBlot-SD transfer buffer.)*
3. Assemble the blot directly on the anode plate of a semi-dry transfer apparatus as indicated below. Remove all air bubbles from the stack by carefully rolling each layer with a roller or test tube.



4. Using a semi-dry transfer apparatus, transfer protein from a standard mini-gel to membrane at a constant current of 1.3 Amps for 5–10 minutes when using a high efficiency blotter or 20–30 minutes if using a standard semi-dry blotter. Using other size gels will require additional optimization.
5. Remove the blot from the apparatus and rinse with high purity water for 5 minutes with gentle agitation.

   Note: FLASHBlot-SD may turn yellow after the transfer is complete. This is normal and does not interfere with downstream applications.