Caution: For Laboratory Use. A research product for research purposes only.

Rapid Western Blotting with the Lightning Blot System

Introduction

Western Blotting of proteins resolved on SDS-PAGE gels is a powerful technique employed in protein analysis (1). Transfer to a solid phase, such as a microporous polyvinylidene fluoride (PVDF) or nitrocellulose membrane can enable protein identification by specific detection reagents, such as antibodies or lectins.

For semi-dry electroblotting, the SDS-PAGE gel (containing resolved proteins) and membrane and paper layers are sandwiched horizontally between two closely separated solid-plate inert electrodes. The paper layers provide a buffer reservoir to supply the needs of the transfer process. These components need to be pre-cut and pre-wet with buffer(s) in advance of the transfer step. Lightning Blot™ Transfer Stacks are provided pre-cut and pre-wet with buffers in 3 vacuum sealed envelopes and can be assembled for semi-dry blotting with very little handling in <2 min. The three buffer system employed in Lightning Blot™ is based on tris-glycine (2) chemistry and is compatible with conventional and fast transfer protocols (3). The PVDF membrane in Lightning Blot™ Transfer Stacks has very low auto-fluorescent background. Lightning Blot™ provides a "ready to blot" solution that is easy to integrate into an existing semi-dry Western blotting process.

1) Pluskal et al, Biotechniques Vol. 4, No. 3, pg 272-282 (1986)
2) Towbin et al, Proc. Natl. Acad. Sci. USA Vol. 76, pg 4350-4354 (1979)
3) Bergendahl et al, J. Immunol. Meth. Vol. 277, Issue 1-2, pg 117-125 (2003)

Product Information (Lightning Blot Mini and Midi Consumables)

Storage Store at room temperature or 4ºC.

Stability The consumables are stable for a minimum of 3 months under proper storage conditions.

Contents Each Lightning Blot Transfer Stack consists of 3 vacuum sealed envelopes containing filter paper or membrane with transfer buffer.

Formats

Mini: 8.0 cm x 8.0 cm. Compatible with standard mini gels such as Bio-Rad Mini-PROTEAN® or Ready Gel® , or Life Technologies™ Novex®, Bolt™, or NuPAGE® Mini.

Midi: 8.5 cm x 13.5 cm. Compatible with standard midi gels such as Bio-Rad Criterion™, or Life Technologies™ NuPAGE® Midi.

Transfer buffers

Envelope 1 (Anode 1): 200 mM Tris base + 10% (v/v) Methanol + filter paper
Envelope 2 (Anode 2): 25 mM Tris base + 10% (v/v) Methanol + PVDF membrane
Envelope 3 (Cathode): 25 mM Tris base + 40 mM Glycine + 10% (v/v) Methanol + filter paper

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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Instructions for Use

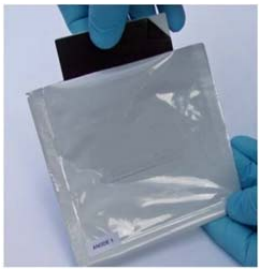
Additional Materials required

- Semi-dry Western blot device (Lighting Blotter or equivalent) for Minigel and Midigel protein transfer
- Power supply able to provide 24V and up to 1000 mA of current.

Protocol for Rapid Semi-dry Western transfer with Lightning Blot™ Transfer Stacks

Prior to transfer, resolve protein samples on an SDS-PAGE gel. Use 1 lane for colored molecular weight markers, such as Multicolor Protein Markers (PerkinElmer catalog number NEL316001EA). Run gel until the tracking dye reached the end. No equilibration of the gel is required before Lightning Blot™ transfer.

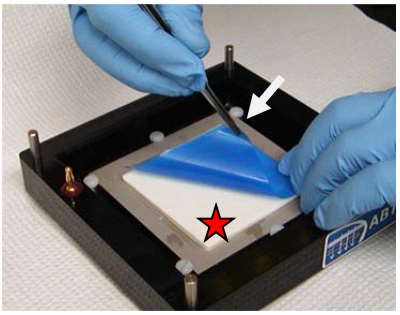
As the dye front approaches the bottom 2 cm of the SDS-PAGE gel cassette, start to assemble the semi-dry transfer device as follows.



1.1. Place the semi-dry transfer device (i.e. Lighting Blotter) on the bench close to the power supply and remove the lid.

1.2. Open Lightning Blot **Envelope 1** using scissors to cut between the two tabs. The black cover sheet will be facing you.

1.3. Place the contents of **Envelope 1** on the base (anode) of the Lightning Blotter and remove the black cover sheet.



1.4 Open Lightning Blot **Envelope 2** using scissors to cut between the two tabs and place the assembly on top of the Anode 1 with the dark blue cover sheet on top. Use the folded corner tab (see arrow) to simplify removal of the cover sheet from the PVDF membrane. This corner (see ★ on Figure 3) can be used to mark the membrane using a pencil for identification.

1.5 When the dye front has reached the bottom of the SDS-PAGE gel cassette, turn off the voltage to the electrophoresis unit and disconnect the electrode connections from the power supply.

Note: If you are using the same power supply for blotting this is a good time to reset the supply to constant 24V, current limit set to 750 mA and time set to 12 min.

1.6 Open the SDS-PAGE gel cassette and prepare the gel to be removed for transfer. Make sure the gel is separated from the cassette to avoid damage during handling.

1.7 Locate the corner folded tab and using forceps remove the blue cover sheet from the PVDF membrane.

1.8 Remove the SDS-PAGE gel from the cassette and place on top of the PVDF membrane. Smooth out any trapped air bubbles.

Note: If the gel is dry and difficult to handle place 1-2 ml of the electrophoresis running buffer on top of the transfer membrane to help prevent any handling problems.

1.9 Open Lightning Blot **Envelope 3** using scissors to cut between the two tabs.

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1.10 Place the contents of **Envelope 3** on top of the gel and remove the light blue cover sheet and smooth out any air bubbles. A short length of glass rod or a pipette can be used as a roller to move from bottom to top (gel) to release any trapped air bubbles.

1.11 Place the upper lid in place and press down lightly to ensure even contact.

1.12 Connect to the power supply and run at a constant 24 v for 12 min. The current will rise to 600 mA within the first 1-2 min but will fall rapidly after 4 min to < 300 mA by the end of the electroblotting step.

Note: Transfer times shorter than 12 minutes (as low as 3 minutes) will lead to some transfer of proteins in the range 10-150KDa but will not adequately transfer higher molecular weight proteins. 12 minutes is a good starting point for optimizing transfer as it achieves efficient transfer of most proteins in the above range with significant transfer of proteins up to 850KDa.

1.13 After 12 minutes shut off the power supply and disconnect the electrodes.

1.14 Remove the upper lid and remove the cathode assembly layers. Remove the SDS-PAGE gel and the PVDF membrane. Confirm that you have transferred the colored molecular weight markers to the membrane. Now, the membrane may be processed for detection of transferred proteins.

Troubleshooting

Technical Support Resources

- **Assay Support Knowledge Base:** **www.perkinelmer.com/ask**
- **Email:** **global.techsupport@perkinelmer.com**
- **Telephone**
  - **USA toll-free** **800-762-4000**
  - **EU toll-free** **00800 33 29 0000**
  - **Finland toll-free** **999 800 33 29 0000**
  - **China toll-free** **800 820 5046**

PROBLEM	REMEDY
Incomplete transfer of colored markers	• Apply power for a full 12 minutes • Check power supply current limit. Normally the current should rise to ~600 mA during the run • Marker proteins above 150 kDa may not transfer completely

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Lightning Blot Ordering Information

Description	Dimensions	Catalog number
Lightning Blotter Mini Transfer System (for 1 miniblot)	10 cm x 10 cm	NEF2000001EA
Lightning Blotter Midi Transfer System (for 1 midiblot or 2 miniblots)	10 cm x 18 cm	NEF201001EA
Lightning Blot Mini Transfer Stack 2pk	8 cm x 8 cm	NEF211001EA
Lightning Blot Mini Transfer Stack 10pk	8 cm x 8 cm	NEF212001EA
Lightning Blot Mini Transfer Stack 50pk	8 cm x 8 cm	NEF213001EA
Lightning Blot Midi Transfer Stack 2pk	8.5 cm x 13.5 cm	NEF221001EA
Lightning Blot Midi Transfer Stack 10pk	8.5 cm x 13.5 cm	NEF222001EA
Lightning Blot Midi Transfer Stack 50pk	8.5 cm x 13.5 cm	NEF223001EA

Related Products for Western Blotting

Multicolored Protein Markers, 8 kDa to 220 kDa	500 µl	NEL316001EA
Western Lightning® Plus Chemiluminescent Substrate for HRP	130 ml	NEL103001EA
Western Lightning® ECL Pro Chemiluminescent Substrate for HRP	130 ml	NEL120001EA
Western Lightning® Ultra Chemiluminescent Substrate for HRP	110 ml	NEL112001EA
Anti-rabbit IgG (goat) HRP	1 mg, 1 mg/ml	NEF812001EA
Anti-mouse IgG (goat) HRP	1 mg, 1 mg/ml	NEF822001EA
Anti-human IgG (goat) HRP	1 mg, 1 mg/ml	NEF802001EA
Streptavidin HRP	2 x 250 µL	NEL750001EA
Anti-fluorescein HRP	2 x 250 µL	NEF710001EA
Anti-digoxigenin HRP	500 µL	NEF832001EA

For complete information on PerkinElmer's products for western blotting, please visit www.perkinelmer.com/western.

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