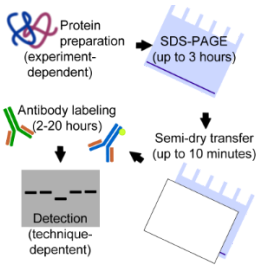
Jun 12, 2020

Semi-dry western blot and chemiluminescent detection



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Protocol materials

- SuperSignal™ West Pico PLUS Chemiluminescent Substrate **Thermo Fisher Scientific** **Catalog #**34577
- Wiper Wypall X60 Quartfold **Kimberly-Clark** **Catalog #**34865
- Immun-Blot® PVDF Membrane 0,2µm **Bio-Rad Laboratories** **Catalog #**1620177
- Glycine **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**50046
- Trizma® base **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T1503-1KG

Troubleshooting

Safety warnings

Methanol: Repeated or prolonged contact with skin may cause dermatitis. The substance may have effects on the central nervous system , resulting in persistent or recurring headaches and impaired vision. Wear safety goggles.

Buffers preparation

1 **Transfer buffer: Alcohol-free Timmons-Dunbar**

Weight reagents as indicated:
- 30.285 g of Tris-Base
- 14.41 g of Glycine

Transfer the reagents to a beaker, add 150 mL of ultrapure water, and stir using amagnetic bar until complete dissolution. Using a volumetric flask, complete the volume to 250 mL with ultrapure water. Store at room temperature.

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Please cite Timmons TM, Dunbar BS. Protein blotting and
immunodetection. Methods Enzymol (1990), 182:679-688. DOI:
10.1016/0076-6879(90)82053-5
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	Timmons-Dunbar 4X Transfer Buffer		
	Reagent	**Concentration**	**Amount**
	Tris-Base	1000 mM	30.285 g
	Glycine	768 mM	14.41 g
	Ultrapure water	q.s.	250 mL

TImmons-Dunbard Alcohol-Free Buffer

Glycine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**50046

Trizma® base **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T1503-1KG

2 **General Washing Buffer: Tris-buffered saline (TBS) 10X**

Weight reagents as indicated:
- 24,2 g of Tris-Base
- 87,6 g of Sodium chloride (NaCl)

Transfer the reagents to a beaker, add 900 mL of ultrapure water, and stir using an magnetic bar until complete dissolution. Using a volumetric flask, complete the volume to 250 mL with ultrapure water. Store at room temperature.

Gradient PAGE Gel preparation

3

Sample preparation and SDS-PAGE

4 Prepare your samples and run SDS-PAGE as usual.

Semi-dry transfer preparation

5 Dilute Timmons-Dunbar Buffer 1X using ultrapure water. Usually, 50 mL for each mini gel (8.4cm x 7cm) to be transfered are enough.

Note

Tip: Perform all steps from this section during SDS-PAGE run

6 Soak 12 WypAll X60 sheets (cutted in gel size) in Timmons-Dunbar Buffer 1X.

Wiper Wypall X60 Quartfold **Kimberly-Clark Catalog #**34865

7 Pre-wet PVDF membrane (cutted in gel size) for 30 seconds in 100% methanol

Immun-Blot® PVDF Membrane 0,2µm **Bio-Rad Laboratories Catalog #**1620177

00:00:30 Membrane activation

8 Discard the methanol and overlay membrane with Timmons-Dunbar Buffer 1X

Note

Membrane will usually float

Semi-dry transfer

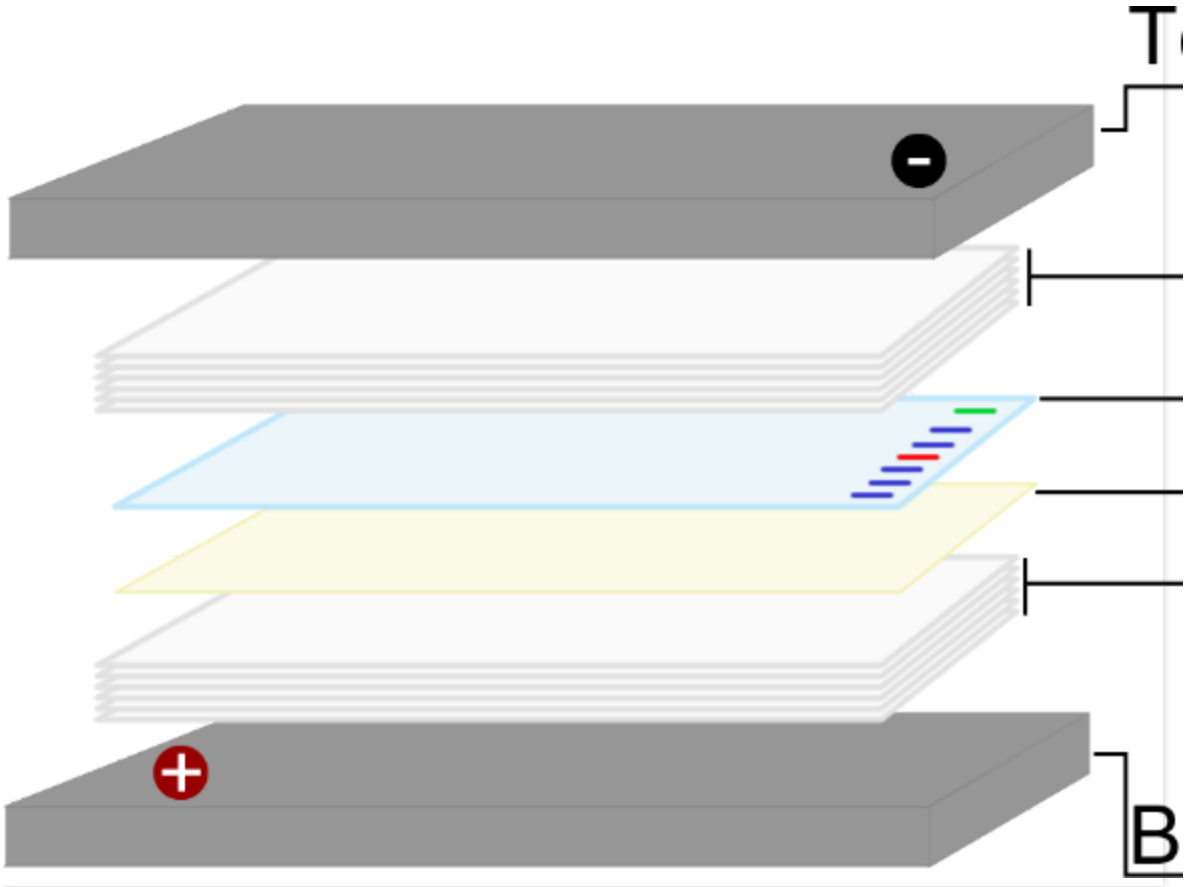
9 Remove gel from electrophoresis chamber and disassembly the sandwich

Note

Do **not** wash the gel! Remaining SDS is important for protein transfer.

10 Carefully and removing bubbles with an roller, assembly the following:
1. Bottom electrode
2. 6 x WypAll X60 buffer-soaked sheets
3. Pre-wet PVDF membrane
4. 6 x WypAll X60 buffer-soaked sheets
5. Top electrode

Follow the picture as an guideline:



11 Perform the transfer at 0.325 mA/mm^2 for 7 minutes when using 0.75mm mini gels.

The following table (From Trans-Blot Turbo Blotting System (Cat #170-4155) manual) is quite usefull:

	Protocol Name	MW (kDa)	Time (min)	2 Mini Gels or 1 Midi Gel	1 Mini Format Gel
	0.75 mm thick gels	> 150	10	2.5 A; up to 25 V	1.3 A; up to 25 V
	Mixed	7			
	< 30	5			
	1.5 mm thick gels	Any	10		

12 Disassembly the sandwich and proceed with blocking and antibody incubation.

Note

Tip: Membranes can be stained with Ponceau S to check protein transfer and binding. Gels can be checked for protein leftover by Coomassie Blue staining.

Blocking and antibody incubation

13 Immediately transfer the membrane to 3% non-fat dry milk diluted in TBS-T (Tris Buffered Saline with 0.05% Tween 20) and incubate with gently rocking for 1 hour.

01:00:00 Membrane blocking

14 Pour off blocking solution and overlay membrane with TBS-T, let it gently rocking for 5 minutes (wash 1/3)

00:05:00 Wash (1/3)

15 Pour off washing solution and overlay membrane with TBS-T, let it gently rocking for 5 minutes (wash 2/3)

00:05:00 Wash (1/3)

16 Pour off washing solution and overlay membrane with TBS-T, let it gently rocking for 5 minutes (wash 3/3)

00:05:00 Wash (1/3)

17 Pour off washing solution and overlay with desired antibody. Incubate from 1 hour (room temperature) up to 16 hours (4°C) with gently rocking.

4 °C for overnight (16h) incubations

Note

Tip: Follow manufacturer's instructions for primary antibody dilution. As a starting point, 1:1,000 dilution in TBS-T with 1% BSA (Bovine Serum Albumin) and 0.02% sodium azide (in order to prevent microorganism growth and antibody reuse).

18 Collect antibody and store at 4°C for further uses. Overlay membrane with TBS-T, let it gently rocking for 5 minutes (wash 1/3).
00:05:00 Wash (2/3)

19 Pour off washing solution and overlay membrane with TBS-T, let it gently rocking for 5 minutes (wash 2/3).
00:05:00 Wash (2/3)

20 Pour off washing solution and overlay membrane with TBS-T, let it gently rocking for 5 minutes (wash 3/3).
00:05:00 Wash (2/3)

21 Pour off washing soution and overlay with horseradish peroxidase conjugated antibody. Incubate from 1-3 hours at room temperature with gently rocking.

Note

Tip: Follow manufacturer's instructions for primary antibody dilution. As a starting point, 1:10,000 dilution in TBS-T.

22 Pour off the secondary antibody. Overlay membrane with TBS-T, let it gently rocking for 5 minutes (wash 1/3).
00:05:00 Wash (2/3)

23 Pour off washing solution and overlay membrane with TBS-T, let it gently rocking for 5 minutes (wash 3/3).
00:05:00 Wash (2/3)

24 Pour off washing solution and overlay membrane with TBS-T, let it gently rocking for 5 minutes (wash 3/3).
00:05:00 Wash (2/3)

Chemiluminescent x-ray film development

25 Mix 1 mL of 'Stable Peroxide Solution' with 1 mL of 'Luminol Enhancer Solution' in a clean container.

SuperSignal™ West Pico PLUS Chemiluminescent Substrate **Thermo Fisher Scientific Catalog #**34577

Note

Tip: Any chemiluminescent reagent can be used

26 Gently transfer the membrane to this container using tweezers and rock for a few seconds.

Note

Tip: Grab the membrane by the border in order to avoid tweezer marks over the bands

27 Transfer the wet membrane to the X-ray cassete. The best approach is 'sandwich' it with two transparent hard plastic foils (as picture bellow).

Note

Tip: X-ray cassetes with white enchancing screens give better results

28 Inside the dark room (remember to left your bright-screen cellphone outside), add one X-ray film over the assembled sandwich and expose for 1 minute.

Note

Tip 1: Increase or reduce exposure time as needed.
Tip 2: Cheaper "hospital grade" X-ray films can be used in place of expensive "research grade" films.

29 Remove the film from cassete and put in a container with diluted autoradiography developer solution and incubate for 30 seconds.

00:00:30 Developer

Note

Tip: Replace solution as it becomes dark

30 Rinse with tap water

31 Put in a container with diluted autoradiography fixer solution and incubate for 1 minute

00:01:00 Fixer

Note

Tip: Replace solution as it becomes yellowish

32 Rinse with tap water and let it dry

go to step #28 If exposure is underoptimal

33 Overlay the dried film over the original cassete sandwich to tick the prestained marker, stick to blank sheet of paper and scan it.