Western blotting

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Western blotting transfers proteins from a gel onto a solid support such as a nitrocellulose or polyvinylidene fluoride (PVDF) membrane. Once transferred, individual proteins, protein–protein or protein–ligand interactions can be detected by probing for specific immunological epitopes or biochemical activity. The choice of the membrane and transfer conditions is largely dependent on the nature of the protein of interest and intended downstream applications.

Wet transfers (*Alternative A*) provide numerous opportunities for optimizing transfer conditions, including time, temperature, voltage, and buffer composition. When carefully tuned, they can achieve almost quantitative transfer for most proteins. Semi-dry transfers (*Alternative B*) are faster and use less buffer. However, they offer fewer opportunities for optimization. If transfer is incomplete, the blotting process cannot simply be extended since by that point, the thin buffer layer is typically exhausted.

The term ‘Western blot’ was coined by W. Neal Burnette (Burnette, 1981) in playful homage to the earlier ‘Southern’ and ‘Northern’ blots for DNA and RNA.

This is a bench card. Full protocol available online.

Procedures

›› *Choosing a Western transfer system*

(1.) Check the literature for transfer conditions of the protein of interest. Epitopes can be sensitive to the way proteins are transferred. Common starting points and optional adjustments:

Sample		Transfer		Transfer buffer			Transfer conditions		
Protein	**State**	**System**	**Membrane**	**Recipe**	**MeOH**	**SDS**	**Voltage**	**Temp.**	**Time**
General	Denat.	Wet	NC, 0.45 µm	Towbin	10%	Omit	100 V	4 °C	30 min
Histone	Denat.	Wet	NC, 0.20 µm	Towbin	20%	Omit	30 V	4 °C	70 min
Histone	Denat.	Wet	PVDF, 0.20 µm	CAPS	Omit	0.01%	100 V	4 °C	60 min
Membrane proteins	Denat.	Wet	PVDF, 0.45 µm	Towbin	20%	0.10%	35 V	4 °C	16 h
Transcription factors	Denat.	Semi-dry	PVDF, 0.20 µm	Bjerrum	10%	0.01%	15 V	25 °C	45 min
Acidic protein	Native	Wet	PVDF, 0.20 µm	Dunn	5%	0.01%	40 V	4 °C	60 min
Basic protein	Native	Wet	PVDF, 0.20 µm	CAPS	5%	0.01%	20 V	4 °C	60 min

(2.) Start by choosing the membrane material, considering the protein’s abundance and hydrophobicity, the detection method, and whether you plan to reprobe the blot.

Membrane	Cost	Binding capacity	Hydrophobicity	Luminescence	Fluorescence	Stripping
PVDF	50 ¢/cm^2	150–200 µg/cm^2	High		Limited	
NC, 0.45 µm	35 ¢/cm^2	80–100 µg/cm^2	Low			Limited

(3.) Once the membrane and transfer system are chosen, fine-tune the methanol and SDS concentrations based on the protein’s size and state.

Transfer			Denatured proteins		Native proteins	
Membrane	**System**	**Protein size**	**MeOH**	**SDS**	**MeOH**	**SDS**
NC	Semi-dry	<20 kDa	20%	Omit	5–10%	0.01%
		20–80 kDa	10%	0.01%	Discouraged	Discouraged
		>80 kDa	10%	0.05%	Discouraged	Discouraged
	Wet	<20 kDa	20%	Omit	5–10%	0.01%
		20–80 kDa	10%	0.05%	Discouraged	Discouraged
		>80 kDa	10%	0.10%	Discouraged	Discouraged
PVDF	Semi-dry	<20 kDa	20%	0.01%	5–10%	0.01%
		20–80 kDa	10%	0.01%	5%	0.01%
		>80 kDa	5%	0.05%	Omit	0.05%
	Wet	<20 kDa	20%	0.01%	5–10%	0.01%
		20–80 kDa	10%	0.05%	5%	0.05%
		>80 kDa	5%	0.10%	Omit	0.10%

(4.) Finally, choose a transfer buffer with a pH above the isoelectric point (pI) of the most basic protein of interest. Towbin buffer is often a good starting point.

Transfer			System		Application
Continuity	**Recipe**	**pH**	**Wet**	**Semi-dry**	
Continuous	Towbin	8.3		Not suitable	Works for most proteins (wet transfer only)
	Bjerrum	9.2			Versatile; preferred for native proteins
	Dunn	9.9		Not suitable	Basic proteins
	CAPS	11.0			Basic proteins or prior to Edman sequencing
Discontinuous	Cathode buffer	9.4	Not applicable		
	Anode buffer	10.4	Not applicable		

›› *Preparing the transfer system*

- ☐ Membrane, PVDF *or* nitrocellulose (NC)
- ☐ Methanol
- ☐ 1 × Transfer buffer, 100 mL

(1.) Freshly prepare the 1 × transfer buffer (or cathode and anode buffer for discontinuous transfers), supplementing methanol or SDS as needed. Bring buffers down to 4 °C.

> ***Critical:*** Do not reuse transfer buffers! Gradient gels always require methanol in transfer buffers to prevent swelling into a trapezium shape during transfer.

(2.) Pre-equilibrate the gel in transfer buffer (or cathode buffer) for 10–20 min before assembly.

(3.) Cut a piece of membrane to the size of the gel. Avoid touching with bare hands.

(4.) *Critical:* Briefly activate PVDF membranes by soaking in methanol, even for methanol-free transfers.

(5.) Hydrate the nitrocellulose or PVDF membrane in transfer buffer (or anode buffer) for 5 min. The membrane will sink on its own once fully soaked.

A › *Wet transfer/Tank transfer*

- ☐ Blotting cassette
- ☐ Blotting paper, 2 mm thick
- ☐ Foam pads
- ☐ Stir bar
- ☐ Transfer tank
- ☐ Ice pack
- ☐ Power supply
- ☐ 1 × Transfer buffer, 1 L

(1.) Soak two sheets of blotting paper in transfer buffer until fully saturated.

(2.) Assemble the gel sandwich in the blotting cassette, submerged in transfer buffer:

Top
Foam pad
Blotting paper
Gel
Membrane
Blotting paper
Foam pad
Bottom

(3.) Use a roller to gently remove air bubbles trapped between the layers of the blot assembly.

(4.) Close the cassette and insert it into the transfer tank with the membrane facing the anode (often red).

(5.) Add a stir bar and ice pack. Fill the tank with transfer buffer, place on a stir plate, and start stirring to ensure even temperature and ion distribution during transfer.

(6.) Connect the power leads and perform the transfer according to your preferred settings. 30–180 min

Transfer	**For 1 h**		**For 3 h**		**For 16 h**	
Recipe	**Voltage**	**Current**	**Voltage**	**Current**	**Voltage**	**Current**
Towbin	50–100 V	200–400 mA	25–50 V	100–200 mA	25–40 V	40–80 mA
Bjerrum	50–100 V	200–400 mA	25–50 V	100–200 mA	25–40 V	40–80 mA
Dunn	40–80 V	200–500 mA	20–40 V	100–250 mA	10 V	40–80 mA

- Run at constant voltage: 100 V for 30 min at 4 °C, *or*
- Run at constant current: 3 mA/cm^2 (Mini Gel, 150 mA; Midi Gel, 300 mA) for 180 min at 4 °C

(7.) Turn off the power supply and disconnect the electrical leads.

B › *Semi-dry transfer*

- ☐ Blotting paper, 2 mm thick
- ☐ Semi-dry blotter unit
- ☐ Power supply
- ☐ R0115 5 × Anode buffer, 1 L
- ☐ R0116 5 × Cathode buffer, 1 L
- ☐ 1 × Anode/Cathode buffer, 100 mL

(1.) If using a discontinuous buffer system, soak one sheet of blotting paper in anode buffer and one in cathode buffer. For continuous systems, soak both sheets in transfer buffer.

(2.) Assemble the transfer stack on the anode plate (typically the bottom electrode):

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Top	
Blotting paper	Cathode buffer
Gel	Cathode buffer
Membrane	Anode buffer
Blotting paper	Anode buffer
Bottom	

(3.) Use a roller to gently remove any air bubbles trapped between the layers.

> ***Quality assurance:*** Bubbles are common between the gel and membrane—press gently but thoroughly. Trapped air can lead to white spots (no transfer) on your final blot.

(4.) Close the semi-dry blotter and connect the electrical leads. Start the transfer according to the manufacturer's instructions.

- Run at constant current: 1.5 mA/cm^2 (Mini Gel, 75 mA) for 15–60 min at room temperature. 15–60 min

(5.) Turn off the power supply and disconnect the electrical leads.

Reversible staining with Ponceau S for total protein normalization

- ☐ Incubation tray *or* Incubation box
- ☐ R0117 0.01% Ponceau S solution, 100 mL

(1.) Carefully remove the membrane from the gel sandwich and place it into a clean incubation tray or box filled with deionized water.

> ***Critical:*** Only handle membranes by the edges with clean forceps. Be careful not to touch the membrane with hands or gloves.

(2.) Decant the water. Add Ponceau S solution until the blot is submerged. Shake for 2–10 min on an orbital shaker (100 rpm) at room temperature.

(3.) Rinse with deionized water to remove the background stain.

(4.) Image the membrane. Quantify total transferred protein for normalization if needed.

(5.) Destain in aqueous buffers or deionized water. Proceed with blocking or detection.

Storing and reusing membranes

(1.) For short-term storage between probing rounds, keep the membrane in TBST or PBS-T at 4 °C. Wrap the container in plastic film to prevent evaporation.

> ***Quality assurance:*** Membranes stored wet should be used within one to two weeks. Extended wet storage increases the risk of microbial contamination and signal degradation.

(2.) Rinse the membrane with deionized water and place it protein side up on a clean sheet of blotting paper. Do not cover the membrane during drying.

> ***Quality assurance:*** Drying preserves protein–membrane interactions and reduces risk of microbial growth. Membranes can be stored dry at 4 °C for months or even years.

(3.) Once dry, place the membrane in a resealable plastic bag or a clean document protector. Label with date and experiment details.

(4.) To reuse, rehydrate the membrane in water for 2 min, then transfer to TBST or another buffer.

> ***Critical:*** For PVDF membranes, pre-wet in 100% methanol for 10–30 s before water to ensure proper rehydration.

List of references

W. Burnette, *Anal. Biochem.* **112**(2), 195—203 (1981).