Western blotting

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.

Risk assessment

- **Methanol damages the optic nerve and central nervous system**
- Methanol is HIGHLY FLAMMABLE
- Methanol is TOXIC if swallowed, inhaled, or in contact with skin
- Work with high voltage power sources

▷ Wear gloves, safety glasses, lab coat

☐ Collect as HAZARDOUS WASTE
☐ DO NOT wash into sewer



Reviewed: Nov 2, 2026

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	60 min
Histone	Denat.	Wet	NC, 0.20 µm	Towbin	20%	Omit	30 V	4 °C	70 min
Large or hydrophobic protein	Denat.	Wet	PVDF, 0.45 µm	Towbin	0–5%	0.10%	30 V	4 °C	16 h
Basic protein (pI > 9)	Denat.	Wet	PVDF, 0.20 µm	CAPS or Dunn	10%	0.01%	100 V	4 °C	60 min

Hint: For most proteins, the defaults will work. For especially large, small, or hydrophobic proteins that transfer poorly under default conditions, adjust membrane pore size, SDS and methanol concentrations, or buffer pH as indicated in the table. The histone conditions follow the Tony Kouzarides lab [Abc23].

(2.) Start by choosing the membrane material, considering the protein's abundance and hydrophobicity, the detection method, and whether the blot will be reprobed.

Membrane	Binding capacity	Hydrophobicity	Luminescence	Fluorescence	Stripping
PVDF	150–160 µg/cm^2	High	✓	Limited	✓
NC, 0.45 µm	80–100 µg/cm^2	Low	✓	✓	Limited

Hint: PVDF binds more protein, making it useful for low-abundance targets. However, as proteins penetrate deeper into PVDF membranes, diffusion may lead to fuzzy bands, especially if the transfer isn't tightly controlled. Soaking the gel in 30% PEG1000 (or higher) for 2 h and transferring in 30% PEG can increase sensitivity 10- to 100-fold [ZSA90].

Hint: While nitrocellulose is too brittle for repeated probing after stripping off the antibodies, it gives lower background in fluorescence applications, and often sharper bands.

(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%	Omit
		20–80 kDa	10%	0.01%	Discouraged	Discouraged
		> 80 kDa	10%	0.05%	Discouraged	Discouraged
	Wet	< 20 kDa	20%	Omit	5–10%	Omit
		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%

Hint: Methanol is thought to improve protein adsorption to nitrocellulose membranes and reduces swelling of polyacrylamide gels in low-ionic strength buffers. Too much methanol, however, may cause protein precipitation and impede transfer. For PVDF membranes, omit methanol where possible to reduce hazardous waste disposal; for nitrocellulose, reduce but do not omit it.

Hint: SDS is optional, but improves the transfer efficiency of proteins larger than 100 kDa or which have a tendency to precipitate. Small proteins or peptides, on the other hand, may completely penetrate through nitrocellulose membranes ("blow-through"). SDS should be omitted in this case.

(4.) Finally, choose a transfer buffer with a pH at least 2 units 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	✓	✓	Works for most proteins
	Bjerrum	9.2	✓	✓	Versatile; preferred for native proteins
	Dunn	9.9	✓	✓	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	✓	

This is why: Buffer pH determines protein mobility during transfer; a protein whose pI is close to the buffer pH barely leaves the gel. Discontinuous transfer uses separate anode and cathode buffer to generate an ion gradient, but these are rarely customized by users.

[KS09]

›› *Preparing the transfer system*

- ☐ Nitrocellulose membrane, pore size 0.20 µm or 0.45 µm
  - *or*: PVDF membrane, pore size 0.20 µm or 0.45 µm
- ☐ Methanol, analytical grade

(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.

This is why: Removing excess SDS and salts minimizes band distortion and blow-through. The gel will settle to its final size.

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

> ***Hint:*** Use a paper trimmer to pre-cut membrane sheets that fit Mini (7.2 cm × 8.6 cm) or Midi Gels (13.3 cm × 8.7 cm).

(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***

- ☐ Nitrocellulose membrane, pore size 0.20 µm or 0.45 µm
  - *or*: PVDF membrane, pore size 0.20 µm or 0.45 µm
- ☐ Blotting paper, 2 mm thick
- ☐ Blotting cassette
- ☐ Foam pads
- ☐ Stir bar
- ☐ Transfer tank
- ☐ Ice pack
- ☐ Roller
- ☐ Power supply
- ☐ 1 × Transfer buffer

(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

> ***This is why:*** Placing the gel on top of the membrane allows you to visually check the alignment and remove air bubbles. The blotting paper protects the gel and membrane from concentrated ions near the electrodes. Too little paper results in lower transfer efficiency and leads to damaged electrode surfaces in the long run.

> ***Hint:*** When transferring multiple gels in one sandwich, interleave them with pieces of water-soaked dialysis membrane.

(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. Run the transfer at one of the following regimes: 60–180 min

- ○ Run at constant voltage: 100 V for 60 min at 4 °C.

  > ***This is why:*** Constant voltage ensures that field strength remains constant, providing the most efficient transfer possible for tank blotting, but generates more heat. Use cooling systems or run at lower voltages to minimize band distortion.

- ○ Run at constant current: about 2.5 mA/cm^2 (Mini Gel, 150 mA; Midi Gel, 300 mA) for 180 min at 4 °C.

  > ***This is why:*** Running at constant current minimizes heat generation, but slows the transfer over time.

> ***Hint:*** Start with 1 h for a new protein; large proteins may need 2 h, small ones less than 1 h. Transfer efficiency and quality generally increase with reduced power and increased transfer time. Use the ranges below as a starting point when switching to other transfer buffers:

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

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

(8.) *Optional:* Stain the gel with Coomassie blue SOP 0010 to verify transfer efficiency.

B › ***Semi-dry transfer***

- ☐ PVDF membrane, pore size 0.20 µm or 0.45 µm
  - *or*: Nitrocellulose membrane, pore size 0.20 µm or 0.45 µm
- ☐ Blotting paper, 2 mm thick
- ☐ Semi-dry blotter unit
- ☐ Roller
- ☐ Power supply
- ☐ 5 × Anode buffer, pH 10.4 (at 1 ×) R0115
- ☐ 5 × Cathode buffer, pH 9.4 R0116
- ☐ 1 × Anode/Cathode buffer

(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.

> ***Hint:*** In most cases, a continuous buffer system like Bjerrum or CAPS is preferred. Discontinuous systems are used for specific commercial protocols and require exact matching of buffer formulations.

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

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

> ***Hint:*** Make sure the membrane is between the gel and the anode side so proteins migrate toward it. The positive electrode (anode) attracts the negatively charged proteins.

(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 the final blot.

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

- ○ For discontinuous anode and cathode buffers, run at constant current: about 0.8 mA/cm² for 60 min at room temperature. 60 min
- ○ For continuous buffers (Bjerrum, Towbin, Dunn, or CAPS), run Mini Gels at 10–15 V for 15–30 min with a current limit of 5.5 mA/cm² (3 mA/cm² for large gels). 15–30 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
- ☐ Forceps
- ☐ 0.01% Ponceau S solution R0117

- Ponceau S is the standard for reversible membrane staining
- Amido black is sometimes reversible under harsh destaining conditions

(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.

> ***This is why:*** Total protein staining with Ponceau S provides a complementary loading and transfer control compared to single housekeeping gene antibodies, which can vary with experimental conditions. Quantify lane intensities to normalize target protein signals across samples.

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

Storing and reusing membranes

- ☐ Plastic sheet protector
  - *or*: Resealable plastic bag

(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:*** Store membranes wet for short periods only, protected from light.

(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:*** For prolonged storage, keep membranes dry at room temperature, protected from light.

> ***Critical:*** Drying can severely decrease the immunoreactivity of blotted proteins; keep membranes that will be reprobed wet [YS09].

(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.

Troubleshooting

Wet transfer/Tank transfer

In Step 2:

– Blurry or wavy bands or lateral smear
  - Ensure gel and membrane are evenly aligned and firmly pressed.
  - Use intact, uncompressed foam pads. Avoid reusing compressed blotting paper.

In Step 3:

– White spots or voids on Ponceau S stain
  - Remove trapped air bubbles by gently pressing the sandwich with a roller or conical tube.
  - Pre-wet blotting paper and pads thoroughly. Degas buffer before use if needed.

In Step 5:

– Uneven transfer efficiency across blot
  - Stir transfer buffer more vigorously during transfer.
  - Use fresh transfer buffer. Cool if appropriate.

In Step 6:

– Faint or absent bands
  - Check transfer time, voltage, and buffer composition. Verify methanol and SDS levels for the protein size.

– No visible transfer on membrane
  - Some membranes, especially PVDF, may have a designated active or coated side; ensure this side faces the gel during assembly.
  - Ensure membrane is between gel and anode.
  - Confirm black lead (cathode) is behind the gel and red lead (anode) is behind the membrane.
  - Verify electrode cables and apparatus function.

Semi-dry transfer

In Step 4:

- Arcing or excessive heat during transfer
  - Check that all components are properly wetted and assembled with no gaps.

Materials

Preparing the transfer system

- ☐ **Membrane**
  - **Nitrocellulose membrane**, pore size 0.20 µm or 0.45 µm
  - *or* **PVDF membrane**, pore size 0.20 µm or 0.45 µm
- ☐ **Methanol**, analytical grade
  CAS 67-56-1

Wet transfer/Tank transfer

- ☐ **Membrane**
  - **Nitrocellulose membrane**, pore size 0.20 µm or 0.45 µm
  - *or* **PVDF membrane**, pore size 0.20 µm or 0.45 µm
- ☐ **Blotting paper**, 2 mm thick
- ☐ **Blotting cassette**
- ☐ **Foam pads**
- ☐ **Stir bar**
- ☐ **Transfer tank**
  - *e.g.* **Mini Trans-Blot® Cell**
    Bio-Rad
- ☐ **Ice pack**
- ☐ **Roller**
- ☐ **Power supply**
  - *e.g.* **PowerPac™ HC**
    Bio-Rad
- ☐ **Transfer buffer**, 1 ×

Semi-dry transfer

- ☐ **Membrane**
  - **PVDF membrane**, pore size 0.20 µm or 0.45 µm
  - *or* **Nitrocellulose membrane**, pore size 0.20 µm or 0.45 µm
- ☐ **Blotting paper**, 2 mm thick
- ☐ **Semi-dry blotter unit**
  - *e.g.* **Trans-Blot® SD Semi-Dry Transfer Cell**
    Bio-Rad
  - *e.g.* **iBlot™ 2 Gel Transfer Device**
    Invitrogen
- ☐ **Roller**
- ☐ **Power supply**
  - *e.g.* **PowerPac™ HC**
    Bio-Rad
- ☐ **Anode buffer**, 5 ×, pH 10.4 (at 1 ×) ⟋ R0115
- ☐ **Cathode buffer**, 5 ×, pH 9.4 ⟋ R0116
- ☐ **Anode/Cathode buffer**, 1 ×

Reversible staining with Ponceau S for total protein normalization

- ☐ **Incubation vessel**
  - **Incubation tray**
  - *or* **Incubation box**
- ☐ **Forceps**
- ☐ **Ponceau S solution**, 0.01% ⟋ R0117

Storing and reusing membranes

- ☐ **Membrane storage container**
  - **Plastic sheet protector**
  - *or* **Resealable plastic bag**

Recipes

Tris-Gly transfer buffer (Towbin), pH 8.3 (at 1 ×), 10 ×

Amount	Ingredient		Stock	Final
30.3 g	Tris base	[77-86-1]	121.14 g/mol	0.25 M
144.1 g	Glycine	[56-40-6]	75.07 g/mol	1.92 M
To 1 L	Water, reagent-grade			

Do not adjust pH. [TSG79]

10 × Tris-Gly transfer buffer (Towbin)
25 mM Tris base, 192 mM Glycine, pH 8.3 (at 1 ×)

No GHS hazards at the indicated concentrations

☐ Dilute below the discharge limit
☐ Flush to drain with excess water

Date: Sign: R0112

Tris-Gly transfer buffer (Bjerrum), pH 9.2 (at 1 ×), 10 ×

Amount	Ingredient		Stock	Final
58.2 g	Tris base	[77-86-1]	121.14 g/mol	0.48 M
29.3 g	Glycine	[56-40-6]	75.07 g/mol	0.39 M
To 1 L	Water, reagent-grade			

Do not adjust pH. ***Note:*** Use for native proteins. [BS86]

10 × Tris-Gly transfer buffer (Bjerrum)

48 mM Tris base, 39 mM Glycine, pH 9.2 (at 1 ×)

No GHS hazards at the indicated concentrations

- ☐ Dilute below the discharge limit
- ☐ Flush to drain with excess water

Date: Sign: R0113

CAPS buffer, pH 11.0, 10 ×

Amount	Ingredient		Stock	Final
22.1 g	3-Cyclohexylamino-1-propanesulfonic acid (CAPS)	[1135-40-6]	221.32 g/mol	100 mM
To 1 L	Water, reagent-grade			

Adjust pH with sodium hydroxide. ***Note:*** Use for basic proteins. [Mat87]

10 × CAPS buffer

pH 11.0



WARNING

Skin and serious eye irritation

- ☐ Neutralise to pH 6–9
- ☐ Flush to drain with excess water

Date: Sign: R0114

Transfer buffer, 1 ×

Amount	Ingredient		Stock	Final
100 mL	Transfer buffer		10 ×	1 ×
100–200 mL	Methanol, analytical grade [67-56-1]	☐	32.04 g/mol	10–20%
	Flammable liquid (H225); Organ toxicity, single exposure (H370, H371)			
2.5 mL	SDS	☐ R0047	20%	0.05%
	Skin irritation (H315); Serious eye damage (H318)			
To 1 L	Water, reagent-grade			

Prepare freshly. Methanol and SDS are optional. ***Critical:*** Methanol must be analytical grade since metallic contaminants will plate on the electrodes and increase the conductivity of the transfer buffer.

1 × Transfer buffer

1 × Transfer buffer, ☐ 10–20% Methanol, ☐ 0.05% SDS

Contains a component outside the substance collection; classify it from its SDS before use.

- ☐ Collect as ORGANIC SOLVENT WASTE
- ☐ Keep away from oxidizers

Date: Sign:

Anode buffer, pH 10.4 (at 1 ×), 5 ×

Amount	Ingredient		Stock	Final
15.1 g	Tris base	[77-86-1]	121.14 g/mol	125 mM
To 1 L	Water, reagent-grade			

Do not adjust the pH; pH 10.4 applies to the 1 × buffer with 20% methanol. [Kyh84]

5 × Anode buffer

pH 10.4 (at 1 ×)



WARNING

Skin and serious eye irritation

- ☐ Neutralise to pH 6–9
- ☐ Dilute below the discharge limit
- ☐ Flush to drain with excess water

Date: Sign: R0115

Cathode buffer, pH 9.4, 5 ×

Amount	Ingredient		Stock	Final
15.1 g	Tris base	[77-86-1]	121.14 g/mol	125 mM
26.2 g	6-Amino-*N*-caproic acid	[60-32-2]	131.17 g/mol	200 mM
To 1 L	Water, reagent-grade			

Adjust pH with sodium hydroxide. [Kyh84]

5 × Cathode buffer

25 mM Tris base, 40 mM 6-Amino-*N*-caproic acid, pH 9.4

No GHS hazards at the indicated concentrations

- ☐ Collect as HAZARDOUS WASTE
- ☐ DO NOT wash into sewer

Date: Sign: R0116

Anode/Cathode buffer, 1 ×

Amount	Ingredient		Stock	Final
20 mL	Anode/Cathode buffer		5 ×	1 ×
10–20 mL	Methanol, analytical grade [67-56-1]	☐	32.04 g/mol	10–20%
	Flammable liquid (H225); Organ toxicity, single exposure (H370, H371)			
0.25 mL	SDS	☐ R0047	20%	0.05%
	Skin irritation (H315); Serious eye damage (H318)			
To 100 mL	Water, reagent-grade			

Prepare freshly. Methanol and SDS are optional. ***Critical:*** Methanol must be analytical grade since metallic contaminants will plate on the electrodes and increase the conductivity of the transfer buffer.

1 × Anode/Cathode buffer

1 × Anode/Cathode buffer, ☐ 10–20% Methanol, ☐ 0.05% SDS

Contains a component outside the substance collection; classify it from its SDS before use.

- ☐ Collect as ORGANIC SOLVENT WASTE
- ☐ Keep away from oxidizers

Date: Sign:

Ponceau S solution, 0.01%

Amount	Ingredient		Stock	Final
10 mg	Ponceau S	[6226-79-5]	760.6 g/mol	0.01%
1 mL	Acetic acid, glacial	[64-19-7]	17.4 M	1.0%
	Skin corrosion (H314)			
To 100 mL	Water, reagent-grade			

Note: The 0.01% solution is as sensitive as a 0.1% solution [SWP+19].

0.01% Ponceau S solution

0.01% Ponceau S, 1.0% Acetic acid

No GHS hazards at the indicated concentrations

- ☐ Hold for EHS review before disposal

Date: Sign: R0117

List of references

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Change log

2026-05-22 **Michael Haugbro, Benjamin C. Buchmuller** Version 1, 2020 to 2026. Initial Western blotting protocol, adapted as an SOP in 2023 with the transfer-buffer selection guide from Carl Roth, "Information Brochure on Transfer Buffers and Parameters", and troubleshooting after Bio-Rad Bulletin 2895 and PhosphoSolutions, "Western Blot Transfer Efficiency".

2026-11-02 **Benjamin C. Buchmuller** Version 2. Transfer conditions default to 30 minute to 1 hour, 35 to 30 volt, and a 0.45 micrometer membrane. Credits: the Michael Knop lab "Tris SDS-PAGE and Western transfer buffers" and the Tony Kouzarides lab for the histone conditions. The CAPS stock reverts to the Carl Roth 10-fold formulation; Ponceau S, the anode buffer, the methanol hazards and the start conditions corrected.

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