Chemiluminescent western blotting procedure

Experimental information

Hypothesis:

Date:

Sample source:

Target protein:

Lysis/extraction buffer:

Electrophoresis conditions

- Gel type and percentage:
- Molecular weight marker:
- Voltage and amps:
- Run time:

Gel layout

Lane	Sample	Total amount loaded
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		

Lane	Sample	Total amount loaded
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		

Find recommended well-loading volumes at **thermofisher.com/wellvolumes**

Materials

- Transfer membrane

  (e.g., Thermo Scientific™ membranes, Cat. No. 88018 or 88518)
- Transfer buffer:
  (e.g., Invitrogen™ NuPAGE™ Transfer Buffer, Cat. No. NP0006; Invitrogen™ Novex™ Tris-Glycine Transfer Buffer, Cat. No. LC3675)
- Wash buffer:
  (e.g., Thermo Scientific™ Pierce™ 20X TBS Tween™ 20 Buffer, Cat. No. 28360; Thermo Scientific™ Pierce™ 20X PBS Tween™ 20 Buffer, Cat. No. 28352)
- Blocking buffer:
  (e.g., Thermo Scientific™ Pierce™ Clear Milk Blocking Buffer, Cat. No. 37587)
- Incubation trays and containers
- Primary antibody
  Antibody target:
  Supplier and Cat. No.:
  Lot number:
- Secondary antibody
  Antibody target:
  Supplier and Cat. No.:
  Lot number:
- Chemiluminescent HRP substrate:
  (e.g., Thermo Scientific™ SuperSignal™ West Pico PLUS substrate, Cat. No. 34580; SuperSignal™ West Atto Ultimate Sensitivity Substrate, Cat. No. A38555)

Find buffer and stock solutions recipes at **thermofisher.com/westernprotocol**

Protocol

1. Prepare transfer buffer for wet or semi-dry transfers based on gel chemistry.
2. Prepare transfer membrane.
   - PVDF: pre-wet in methanol or ethanol (100%) for 30 seconds, briefly rinse in deionized water, and equilibrate in transfer buffer for 5 minutes.
   - Nitrocellulose: equilibrate directly in transfer buffer for 5 minutes.
3. Prepare gel for transfer by rinsing the gel in water for 1-5 minutes to remove any SDS.
4. Follow manufacturer's instructions for wet, semi-dry, or dry transfer.
   Transfer method:
   Transfer device:
   Voltage and program:
   Transfer time:
   Additional transfer notes:
5. After protein transfer, wash the membrane in deionized water 4 times for 5 minutes each with agitation to remove all transfer buffer.
   Wash 1 Wash 2 Wash 3 Wash 4
6. Incubate the membrane with a sufficient volume of blocking buffer for 30–60 minutes at room temperature with agitation.
   Blocking buffer incubation time:
7. Dilute the primary antibody per supplier recommendations in the blocking buffer.
   Primary antibody dilution:
   Antibody stock concentration:
8. Incubate the membrane protein-side up in the primary antibody solution with agitation, for 1 hour at room temperature or overnight at 2–8°C. Ensure the volume of the antibody solution is enough to fully cover the membrane.
   Incubation time:
   Incubation temperature:
9. Wash the membrane 3 times with agitation for 10 minutes each in wash buffer.
   Wash 1 Wash 2 Wash 3
10. Dilute the conjugated secondary antibody in an appropriate volume of wash buffer or alternatively in blocking buffer.
    Secondary antibody dilution:
    Antibody stock concentration:
11. Incubate the membrane protein-side up in the secondary antibody solution for 1 hour with agitation at room temperature. Ensure the volume of the antibody solution is enough to fully cover the membrane.
12. Wash the membrane 6 times with agitation for 5 minutes each in wash buffer to remove any unbound secondary antibodies. It is crucial to thoroughly wash the membrane at this step.
    Wash 1 Wash 2 Wash 3
    Wash 4 Wash 5 Wash 6
13. Prepare the working solution of chemiluminescent substrate based upon the manufacturer's instructions. Suggested volume is (0.1 mL working solution per cm^2 of membrane.
14. Incubate the blot with the working solution for 1 minute when using standard ECL substrates or 5 minutes when using high-performance substrates, such as Thermo Scientific™ SuperSignal™ substrates.
15. Using tweezer, remove the blot from the working solution and drain excess reagent.
16. Place the blot in clear plastic wrap or a sheet protector and remove bubbles by rolling with a blot roller or a pipette.
17. Image the blot.

    Imaging system or developer:
    Exposure time:
    File name:
    File location:

Table 1. Recommended primary and secondary antibody dilutions to use with Thermo Scientific™ chemiluminescent substrates.

	Recommended primary antibody dilution	Recommended secondary antibody dilution
Pierce ECL	1:1,000 (0.2–10 µg/mL)	1:1,000–1:15,000 (0.07–1.0 µg/mL)
SuperSignal West Pico PLUS	1:1,000 (0.2–1.0 µg/mL)	1:20,000-1:100,000 (10–50 ng/mL)
SuperSignal West Dura	1:5,000 (0.02–1.0 µg/mL)	1:50,000-1:250,000 (4–20 ng/mL)
SuperSignal West Femto	1:5,000 (0.01–0.2 µg/mL)	1:100,000-1:500,000 (2–10 ng/mL)
SuperSignal West Atto	1:5,000 (0.2–1.0 µg/mL)	1:100,000-1:250,000 (4–10 ng/mL)

Results and observations

Future direction and next steps

Reviewed by:

Date reviewed:

Find additional resources at
thermofisher.com/westerneducation

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