Fluorescent western blotting procedure

Experimental information

Hypothesis:

Date:

Sample source:

Target proteins:

Lysis/extraction buffer:

Electrophoresis conditions

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

Gel layout

Lane	Sample	Total amount loaded	Lane	Sample	Total amount loaded
1			14		
2			15		
3			16		
4			17		
5			18		
6			19		
7			20		
8			21		
9			22		
10			23		
11			24		
12			25		
13			26		

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

Materials

- Transfer membrane

  Membrane type

  Membrane pore size

  (e.g., Thermo Scientific™ membranes, Cat. No. 88018 or 22860, or equivalent)

- 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)
- Filtered blocking buffer:
  (e.g., Thermo Scientific™ Blocker™ FL Fluorescent Blocking Buffer, Cat. No. 37565)
- Incubation trays and containers
- Primary antibodies
  Antibody target 1:
  Supplier and Cat. No.:
  Lot number:

  Antibody target 2:
  Supplier and Cat. No.:
  Lot number:
- Secondary antibodies
  Antibody conjugate:
  Antibody target:
  Supplier and Cat. No.:
  Lot number:

  Antibody conjugate:
  Antibody target:
  Supplier and Cat. No.:
  Lot number:

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 antibodies per supplier recommendations in the blocking buffer.
   Primary antibody 1 dilution:
   Primary antibody 2 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. (If using a fluorescently conjugated primary antibody, proceed to step 11.)

   ☐ Wash 1 ☐ Wash 2 ☐ Wash 3

10. Prepare dilutions of the conjugated secondary antibody ranging from 0.4 to 0.1 µg/mL in an appropriate volume of wash buffer, or alternatively in blocking buffer. From a 2 mg/mL antibody stock, dilute 1:5,000 to 1:20,000:

    - **1:5,000:** 3 µL of secondary antibody in 15 mL wash buffer

    - **1:10,000:** 1.5 µL of secondary antibody in 15 mL wash buffer

    - **1:20,000:** 0.75 µL of secondary antibody in 15 mL wash 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. Protect the membrane from bright light to prevent photobleaching of the fluorescent dyes.

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. Protect the membrane from bright light to prevent photobleaching of the fluorescent dyes.

    ☐ Wash 1 ☐ Wash 2 ☐ Wash 3
    ☐ Wash 4 ☐ Wash 5 ☐ Wash 6

13. Blots can be imaged immediately while still wet, or alternatively may be dried prior to imaging. To prevent contamination, place the blot in a sheet protector or on a clean surface prior to imaging. Image the blot.
    Imaging system:
    Exposure time:
    File name:
    File location:

Results and observations

Future direction and next steps

Reviewed by:

Date reviewed:

Find additional resources at
thermofisher.com/westerneducation

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