



# Western Blotting Reagents and Protocols

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Western blotting is a method used to detect specific proteins using antibodies after separation of a protein mixture via SDS-PAGE and transfer to a membrane. There are two types of transfer devices: tank-type and semi-dry type. The semi-dry type is more commonly used because of its high transfer efficiency and short run time.

### [Solution Preparation]

Prepare the following buffers:

- Transfer Buffer (1000 mL)

Reagent	Amount (Final Concentration)
<a href="#">Glycine</a> (TCI Product No. <b>G0317</b> )	14.4 g (192 mM)
<a href="#">Tris-Base</a> (TCI Product No. <b>T2516</b> )	3.0 g (25 mM)
<a href="#">Methanol</a> (TCI Product No. <b>M0628</b> )	200 mL

Dissolve in deionized water and top up to 1 L (no pH adjustment required)

- Blocking Buffer (1000 mL)

Reagent	Amount (Final Concentration)
10x PBS	100 mL
<a href="#">Polyoxyethylene Sorbitan Monolaurate</a> (TCI Product No. <b>T2530</b> )	1 mL (0.1%)
BSA	10 g (1%)
Deionized Water	900 mL

- PBST (1000 mL)

Reagent	Amount (Final Concentration)
10x PBS	100 mL
<a href="#">Polyoxyethylene Sorbitan Monolaurate</a> (TCI Product No. <b>T2530</b> )	1 mL (0.1%)
Deionized Water	900 mL

### [Transfer/Antibody Reaction]

1. After briefly immersing a PVDF membrane in methanol for approx. 1 minute, allow to soak in transfer buffer. If using nitrocellulose membrane, methanol activation is not required, simply soak in transfer buffer.
2. Carefully stack filter paper, membrane, and post-electrophoresis gel in the chamber of a semi-dry type transfer equipment as indicated below, taking care not to introduce air bubbles between layers.

- Electrode
3 pieces of filter paper (moistened with transfer buffer)
Post-Electrophoresis Gel
Membrane (PVDF or Nitrocellulose)
3 pieces of filter paper (moistened with transfer buffer)
+ Electrode

3. Run for 1 hour at a current of 2 mA per 1 cm<sup>2</sup> gel.
4. Immerse transferred membrane in blocking buffer and incubate at room temperature for 1 hour (or overnight at 4°C).
5. Discard blocking buffer and wash membrane (1–2 times with PBST, approximately 5 minutes each).
6. Immerse membrane in appropriately-diluted solution of primary antibody (diluted with PBST) and incubate at room temperature for 1-2 hours (or overnight at 4°C).
7. Wash three times with PBST (approximately 5 minutes each wash) to thoroughly remove unbound primary antibody.
8. Immerse membrane in appropriately-diluted secondary antibody solution (diluted with PBST) and incubate at room temperature for approximately 1 hour.
9. Wash three times with PBST (approximately 5 minutes each wash) to thoroughly remove unbound secondary antibody.

## [Detection]

### Detection Reagents:

- [DAB staining Kit](#) (TCI Product No. **D5909**)
- [Chemiluminescence HRP Substrate Solution Kit \[for Western Blotting\]](#) (TCI Product No. **C4087**)

### Procedure:

#### Procedure of DAB staining Kit (TCI Product No. **D5909**)

1. Prepare reaction solution by adding 5 drops of provided DAB solution and 2 drops each provided hydrogen peroxide solution and 1.5M Tris-HCl buffer to 5 mL of deionized water.
2. Immerse the membrane washed in PBST into the reaction solution and shake until the band of the target protein develops color.
3. Remove the reaction solution, wash with deionized water, and photograph the membrane.

#### Procedure of Chemiluminescence HRP Substrate Solution Kit (TCI Product No. **C4087**)

1. Bring Chemiluminescence HRP Substrate Solution Kit to room temperature.
2. Mix equal volumes of included Solution A and Solution B.  
(use 0.1 mL of mixture per 1 cm<sup>2</sup> of membrane)
3. After tapping edge of membrane on tissue to thoroughly remove excess PBST, place membrane on plastic wrap or transparent polyethylene sheet.
4. Apply mixture drop-by-drop over entire membrane.
5. Incubate at room temperature for 60 seconds, making sure to remove mixture from membrane thoroughly after incubation.
6. Place membrane on fresh plastic wrap or polyethylene sheet.
7. Photograph using a chemiluminescence imaging system.

### **When reacting with other antibodies after chemiluminescent detection**

### Detection Reagents:

- [Western Blot Stripping Buffer \[for Biochemical Research\]](#) (TCI Product No. **W0024**)

### Procedure:

1. After detection, wash membrane twice with PBST (10 minutes each).
2. Immerse membrane in enough stripping buffer to submerge and shake gently at room temperature for 30 minutes.  
\*Depending on the specific antigen/antibody affinity strength, antibody may not be completely removed from the membrane.
3. Wash membrane three times with PBST (10 minutes each).
4. Re-block and perform second antigen-antibody reaction.