

OneStep Western Blocker and Signal Enhancer (Protein-Free)

PRODUCT DESCRIPTION

OneStep Western Blocker and Signal Enhancer (Protein-Free) is a blocking solution for Western blot analysis. It not only provides blocking and primary and secondary antibody hybridization in one step, but also enhances the signal developed with HRP (horseradish peroxidase) or AP (alkaline phosphatase) substrates. Therefore, it serves as both blocker and enhancer in Western analysis. With the three-in-one step procedure, OneStep Blocker is an ideal alternative solution for the time consuming and laborious Western procedure.

FEATURES

- ▶ **3 steps in one:** Block the membrane and dilute 1° Ab & 2° Ab in one step.
- ▶ **Enhance antibody signal:** It shows a two- to five-fold increase in signal intensity for most protein targets, enabling much less protein to be detected with the same substrate and method.
- ▶ **Timesaving:** It saves hours in the antibody detection process during the Western Blot, with only one hour needed.
- ▶ **Universal antibody diluent:** Ready-to-use dilution buffer for most 1° & 2° Ab.
- ▶ **No blocking step needed:** Simply immerse the membrane in the OneStep Blocker solution with your antibodies.
- ▶ **Protein free:** Reduces overall background and minimizes non-specific signals often seen with ECL detection.
- ▶ **Fewer hands-on steps:** Three wash steps are not required, meaning no need to transfer the membrane in and out of the container.
- ▶ **Effective with any ECL:** After the antibody detection process, the signal can be developed with both HRP (horseradish peroxidase) and AP (alkaline phosphatase) substrates.
- ▶ **Compatible with PVDF & NC membrane:** Regardless of the pore size, the OneStep Blocker minimizes the background from non-specific protein binding by antibodies.
- ▶ **Improve protein detection:** Improve the binding process of target proteins so that specific antibodies can bind more effectively.

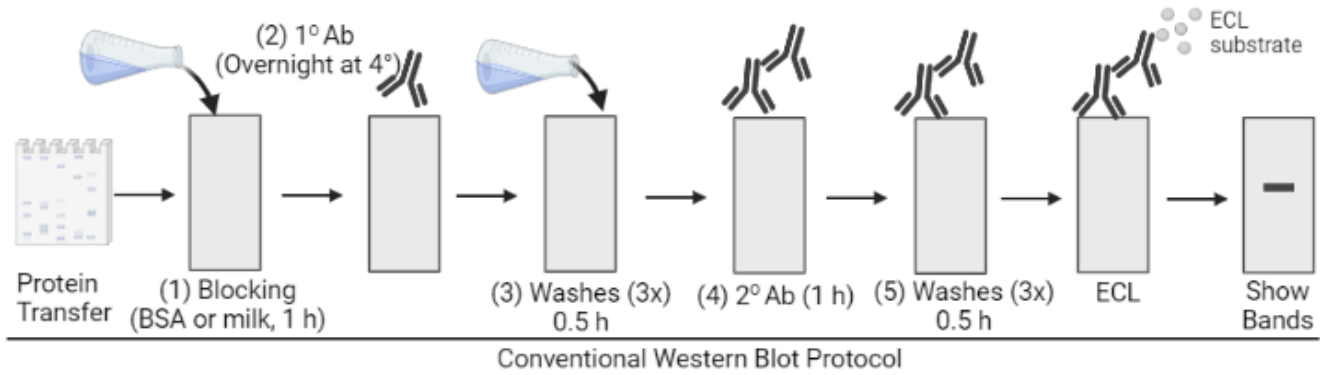
STORAGE CONDITIONS

Stable for up to 24 months at room temperature.

REQUIRED MATERIALS (NOT PROVIDED)

- Primary antibody.
- Secondary antibody conjugated with HRP.
- Wash buffer: PBST (*PBS with Tween-20*) or TBST (*Tris buffered saline with Tween-20*) buffer.
- ECL (*Enhanced chemiluminescence*) or colorimetric reagents.
- Shaker: orbital or rocking shaker.

► **DATASHEET: OneStep Western Blocker and Signal Enhancer (Protein-Free)**



MP Biomedicals OneStep Western Blocker and Signal Enhancer (Protein-Free) is the Next-Generation Reagent to Simplify Western Procedures: Save Time & Enhance ECL Signal.

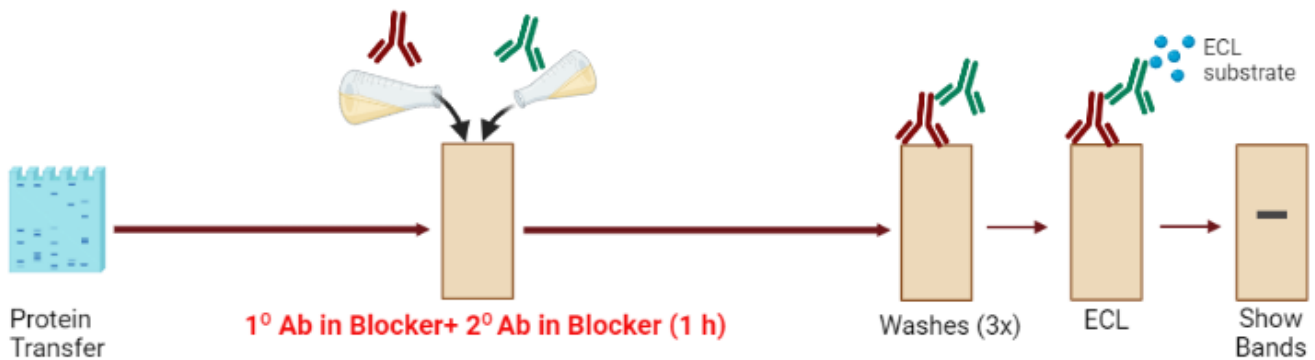


Figure 1. The comparison between MP Biomedicals' OneStep Blocker-based approach and conventional Western procedure.

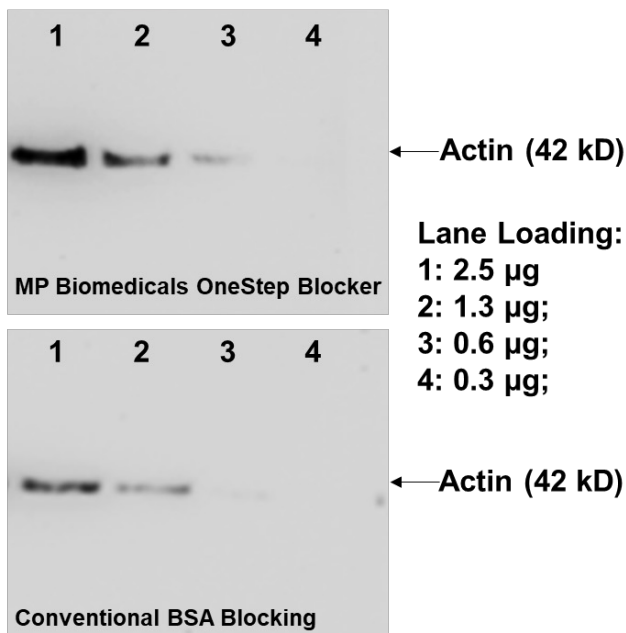


Figure 2. Western blotting of HeLa cell lysates with different types of blocking reagents, using actin rabbit monoclonal antibody (1 µg/mL) and secondary goat anti-rabbit HRP (1:5,000).

PROTOCOL

- 1** After Western blot transferring, immerse the PVDF or NC membrane in PBST/TBST buffer for 5 minutes.
- 2** Dilute the primary antibody and secondary antibody with proper amounts of OneStep Blocker.
 - 2.1** For example, when the dilution factor for both primary and secondary antibodies is 1: 10,000, add 2 μ L of the primary antibody to 10 mL of the OneStep Blocker (1st tube), followed by adding 2 μ L of the secondary antibody to another 10 mL of the OneStep Blocker (2nd tube).
 - 2.2** Thoroughly mix the antibody-OneStep Blocker solution inside each tube by inverting it back and forth.
 - 2.3** Pour the primary antibody-OneStep Blocker solution into the prepared container first, followed by the addition of the secondary antibody-OneStep Blocker solution into the same container.
- 3** Incubate the membrane immediately in the antibody-OneStep Blocker solution at room temperature for 1–2 hours with gentle agitation. Please note that after mixing the primary and secondary antibodies, the membrane needs to be immediately immersed in the mixture within 10 minutes for optimal performance.
- 4** Wash the membrane with PBST/TBST three times with shaking.
- 5** Drain excessive wash buffer and immediately perform image development methods with ECL or colorimetric system.

IMPORTANT NOTES

- Please note that after mixing the primary and secondary antibodies, the membrane needs to be immediately immersed in the mixture within 10 minutes for optimal performance.
- The dilution for the secondary antibody should be at least 1:10,000. A higher level of background noise will be observed with a high concentration of secondary antibody.
- Do not incubate membrane in OneStep Blocker for over 4 hours to avoid high background. Overnight incubation is not recommended.
- When the antibody concentration is too high or prolonged incubation takes place, it may cause high background. When excessive background occurs, try the following:
 - Reduce/optimize primary and/or secondary antibody concentrations.
 - Use dot-blot test to optimize antibody concentrations.
 - Reduce/optimize incubation time.



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