— PROTOCOL —

OneStep Blocker
– Western Blocking Solution and Signal Enhancer

Catalog Number	Unit Size	Reactions
BS001-B020ML	20 ml	Sufficient for over one mini-gel size membrane.
BS001-B500ML	500 ml	Sufficient for over 25 mini-gel size membranes.

Storage : 4℃, up to one year.

Description

OneStep Blocker is a blocking solution for Western blot analysis. This **OneStep Blocker** buffer 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. It, therefore, serves as both blocker and enhancer in Western analysis. With the three-in-one step procedure, **OneStep Blocker** is a time and labor economic solution for the time consuming and laborious Western procedure.

Quality control

The quality of this product is tested on a lot-to-lot basis to ensure consistent quality.

Required material and equipment not provided

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

Protocol

1. After Western blot transferring, immerse the PVDF or NC membrane in PBST 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 2ul of the primary antibody to 10ml of the **OneStep Blocker** (1st tube), followed by adding 2ul of the secondary antibody to another 10ml of the **OneStep Blocker** (2nd tube).

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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 obtaining the optimal performance.
4. Wash the membrane with PBST/TBST three times with shaking.
5. Drain excessive wash buffer and perform image development methods with ECL or colorimetric system immediately.

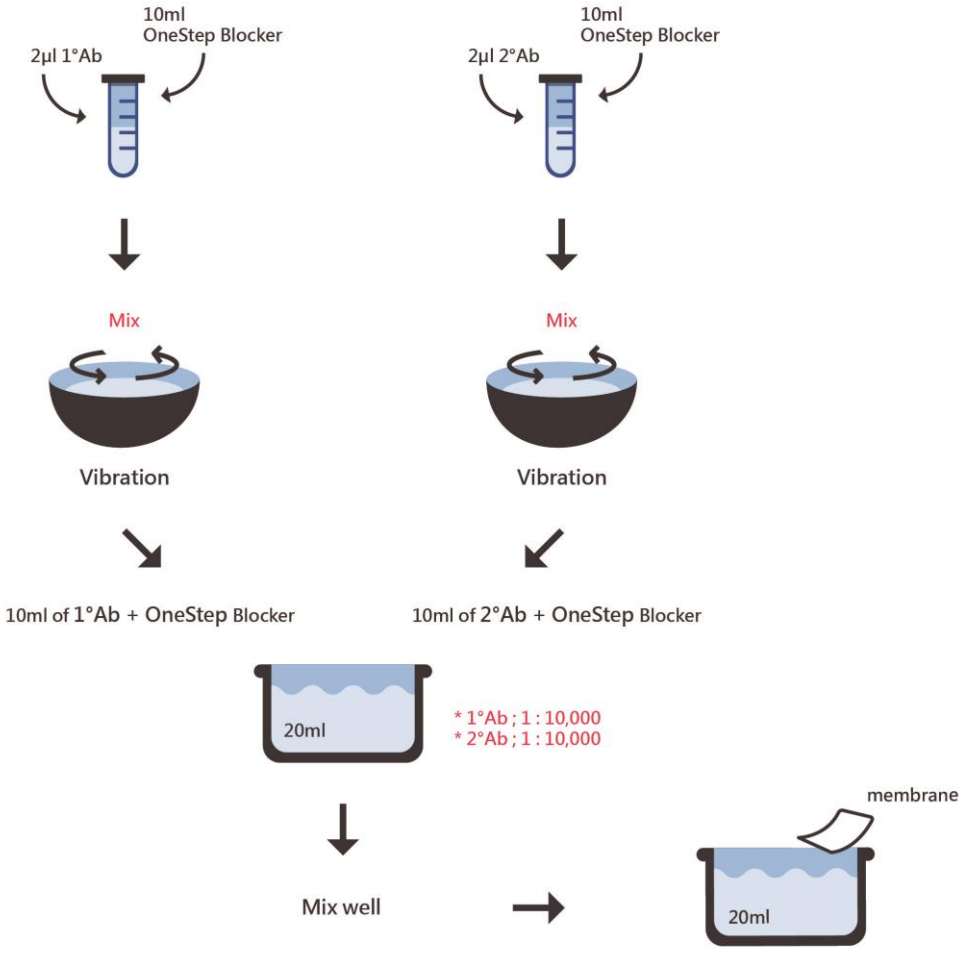

Figure 1. A simple flowchart shows diluting primary and secondary antibodies with proper amounts of OneStep Blocker, based on the 1:10,000 dilution factor for both primary and secondary antibodies.

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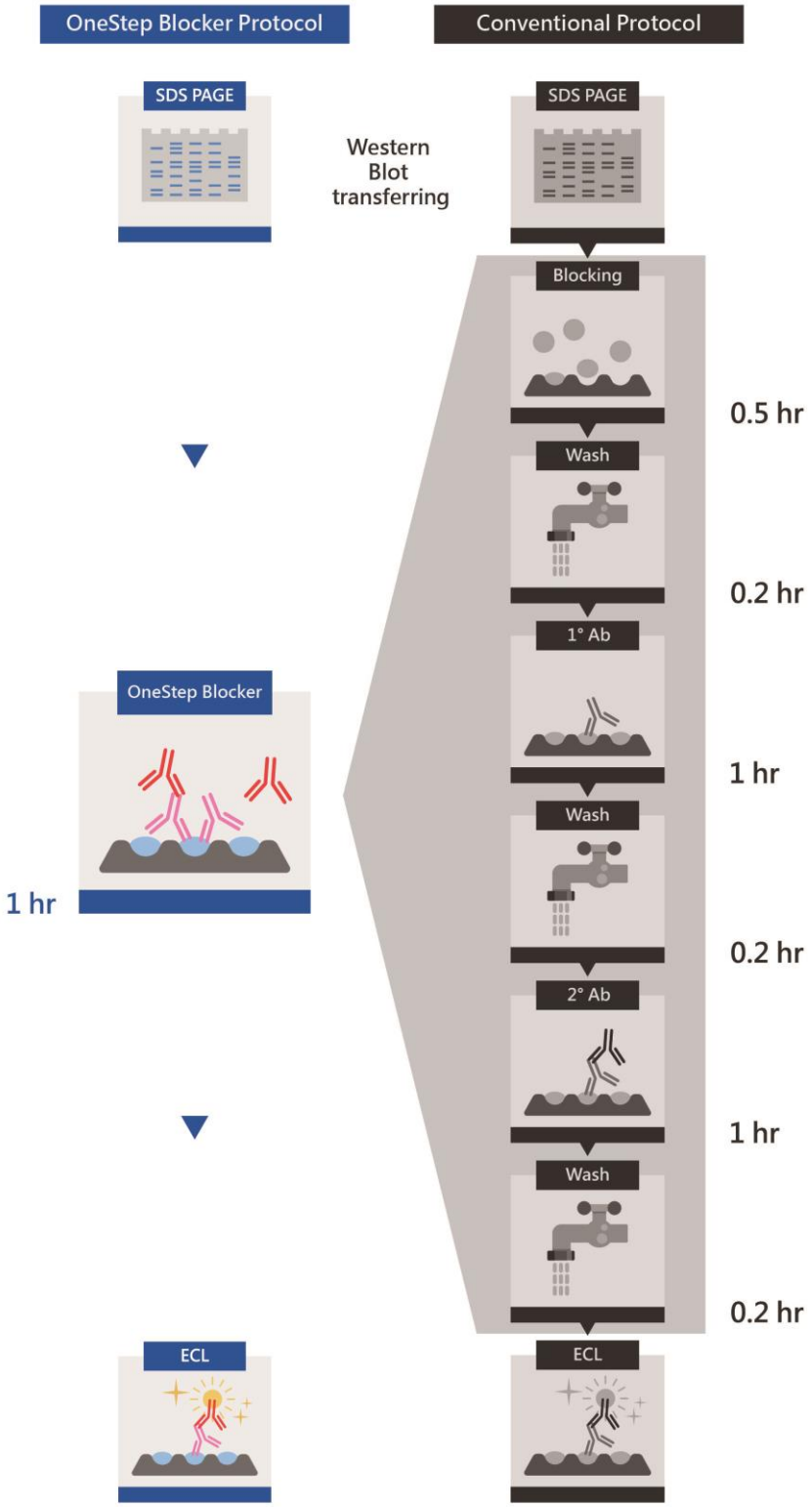

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User's Note

(1) Please note that after mixing the primary and secondary antibodies, the membrane needs to **be immediately immersed in the mixture within 10 minutes** for obtaining the optimal performance.

(2) The dilution for the **secondary antibody** should be **at least 1:10,000 or more.** A higher level of background noise will be observed as a result with a high concentration of secondary antibody.

(3) Do not incubate membrane in **OneStep Blocker** for over 4 hours to avoid high background. Overnight incubation is especially not recommended.

(4) The primary/secondary antibodies mixed in **OneStep Blocker** solution may be reused within 3 days. Enhancing effect may trail off along with the increasing storage time or repetitiveness. Keep the mixed solution refrigerated. For critical experiment or strong signal, fresh preparation of antibody-**OneStep Blocker** solution is required.

(5) When the antibody concentration is too high or if the prolonged incubation takes place, it will cause high background. When excessive background occurs, please try the followings:
   (a) Reduce/optimize primary and/or secondary antibody concentrations.
   (b) Use dot-blot test to optimize antibody concentrations.
   (c) Reduce/optimize incubation time.

Cautions

1. This product may be shipped at ambient temperature but should be stored between 2~8℃. The effective period is within a year.
2. For research use only. Not intended for any animal or human therapeutic or diagnostic uses.

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