Western Blocking Buffer (Casein in PBS)

Introduction

Western Blocking Buffer (Casein in PBS) is a milk protein purified by the Hammarsten method, used for blocking excess binding sites in ELISA, Western blot, immunohistochemistry, and other immunochemical applications. Western Blocking Buffer (Casein in PBS) is formulated in phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) containing 1% (w/v) casein. Compared to serum- or milk-based blocking buffers, this single purified protein offers fewer chances of cross-reactivity with assay components. Western Blocking Buffer (Casein in PBS) is an excellent alternative when high background or antigen/antibody masking occurs with non-fat milk blockers.

Protocol

■ Block western blots:

1. After protein transfer, remove the membrane from the transfer apparatus and wash in deionized water for 5 minutes with agitation to remove all transfer buffer.
2. Add sufficient Western Blocking Buffer (Casein in PBS) to cover the membrane.
3. Incubate with shaking for 30 minutes to 2 hours at room temperature.
4. Proceed with the Western blot protocol suitable for downstream detection.

■ Block ELISA plates:

1. Coat ELISA plates with antigen or antibody.
2. Add 300 µL of Western Blocking Buffer (Casein in PBS) to each well, then incubate the plate for 30 minutes to 2 hours at room temperature or 37°C.
3. Empty the plate by aspiration or inversion.
4. Proceed with the ELISA protocol suitable for downstream detection.

***Note:** For optimal results when drying antigen- or antibody-coated microplates, use a protein stabilizer.

Note

1. Milk protein-based products may contain biotin that interferes with streptavidin and avidin systems. When using streptavidin and avidin enzyme conjugates, use BSA or blocking buffers free of endogenous biotin.
2. Empirical testing is essential to determine the appropriate blocking reagent for your system. Proper blocking

reagents can increase sensitivity and prevent non-specific signals caused by cross-reactivity between antibodies and blocking reagents.

3. Use the provided Western Blocking Buffer (Casein in PBS) for initial tests. However, other concentrations may be beneficial for specific systems.
4. Adding a final concentration of 0.05% Tween-20 detergent to Western Blocking Buffer (Casein in PBS) may improve blocking performance; however, it is not required or recommended for all systems. Use only high-quality products, such as specially purified Tween-20 detergent that is free of peroxides and carbonyl groups that may interfere with some systems.
5. Western Blocking Buffer (Casein in PBS) diluted to 0.05% (w/v) casein containing 0.05% Tween-20 detergent can be used as an antibody diluent to improve the signal-to-noise ratio.
6. Storage conditions: Store at 4°C.
7. This product is for scientific research use only.

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