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TECHNICAL DATA SHEET

Casein Blocking Solution in TBS
C44000

CAS Number: 7732-18-5; 9000-71-9; 1185-53-1; 7647-14-5

Solubility: Water

Storage Temperature: -20°C

Contains 3% Casein in TBS buffer. Optimized for use in western blotting to minimize non-specific binding of antibodies.

Blocking non-specific binding sites on the membrane is crucial before detecting the target protein(s) on the Western blot. This step ensures that antibodies bind exclusively to their intended targets. The process involves incubating the membrane in a blocking buffer containing a blocking agent like Casein. Specifically, a Casein (3%) blocking buffer in TBS (Tris Buffered Saline) is utilized, which is a purified solution of casein protein designed for blocking steps in Western blot or ELISA assays.

Note: Because casein is a phosphoprotein, it is not recommended when the protein of interest is a phosphoprotein as target antigen.

Adding a small amount of Tween-20 detergent to blocking solutions can reduce background staining.

Procedure:

A. SDS-PAGE & Transfer of Proteins:

Following the separation of proteins by SD-PAGE, the transfer of these proteins from the gel to a membrane is conducted. This process involves applying an electrical field oriented perpendicularly to the gel's surface. As a result, negatively charged proteins migrate out of the gel and onto the membrane. There are two primary types of membranes used for this purpose: PVDF (polyvinylidene difluoride) and nitrocellulose.

B. Visualization of Proteins Transfer:

Once the proteins have been transferred onto the membrane, it's essential to confirm the success and efficiency of the transfer process. This can be achieved by visualizing the proteins on the membrane using Ponceau S stain. The following protocol outlines how to carry out this visualization:

1. Wash the membrane in PBST (PBS- with Tween-20) or TBST (TBS- with Tween-20).
2. Incubate the membrane with Ponceau S stain (0.1% Ponceau S and 5% acetic acid in deionized water) , agitate 10-15 min.
3. Wash membrane repeatedly by washing in TBST or PBST with water until the protein bands are well-defined.

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C. Blocking of Membranes::

Blocking non-specific binding sites on the membrane is a crucial step before detecting the target protein(s) on the Western blot. This ensures that antibodies exclusively bind to their intended target protein, enhancing the accuracy and specificity of the results. This step is performed by incubating the membrane in a blocking buffer-containing blocking agent.

BSA (Bovine Serum Abumin, SKU A30075) or casein, or non-fat dried milk (SKU M17200) are commonly used as blocking agents. Following procedure is used for blocking the membrane.

Incubate membrane with Blocking Buffer containing 1 % Casein in TBS (Tris-buffered Saline: 50 mM tris-HCl and 150 mM NaCl) for 2 hours at RT or overnight at 4°C, with constant rocking action.

Note:

1. To reduce the concentration of Casein from 3% to a final concentration of 1%, dilute it threefold with 1X TBS. For example, add 20 ml of 1X TBS to 10 ml of the blocking solution containing 3% Casein.

2. Tween-20 is commonly used as a surfactant to block protein non-binding sites on the membrane. It aids in rinsing away unbound antibodies and diminishing the non-specific binding of antibodies to antigens. This process contributes to reducing background noise and improving signal strength through the specific binding of antibodies to antigens.

3. Under normal conditions, the final concentration of Tween-20 in the blocking agent should be between 0.05% to 0.1%. The higher concentration of Tween-20 in the blocking solution containing Casein as a blocking agent tends to increase the size of the casein micelles or the aggregation of casein [Titapiccolo et.al., 2010].

Refernce: Titapiccolo, G.I., Corredig, M., and Alexander, M. (2010). Modification to the renneting functionality of casein micelles caused by nonionic surfactants. Journal of Dairy Science. 93 (2): 506-514 DOI:https://doi.org/10.3168/jds.2009-2629