BIOLOGICAL INDUSTRIES
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EZ-Block

Cat. No.: 41-805-10 (10 grams)

Storage: Room Temperature

Product Description

EZ-Block is used in hybridization and detection procedures using non-radioactive nucleic acid probes, and for Western blots.

When immunoassays and hybridization assays, such as dot blots, Western blots, Southern blots, or Northern blots are performed, there is nonspecific binding resulting in high background. In order to reduce the nonspecific binding, EZ- Block reagent is used to "block" unbound sites left after immobilization of the specific protein or after the hybridization with non-radioactive probe. EZ-Block improves sensitivity and reduces background.

Note: Nonfat dry milk inhibits the streptavidin-biotin interaction due to its content of biotin

1. Procedure

Proteins:

For blotting applications such as Western blots and dot blots, add 0.2% (w/v) EZ-Block into TBST or PBST, heat to 75-80°C in a water bath or microwave oven, and stir well until dissolved. EZ-Block dissolves to give a milky solution. Use for blocking and for dilutions of antibodies.

Note: Do not use EZ-Block in PBST for alkaline phosphatase conjugate dilutions

Nucleic Acids:

For hybridization applications add 0.2% (w/v) EZ-Block to Tris-Saline buffer (100mM Tris-Cl pH 7.5, 600mM NaCl), heat to 60-65°C in a water bath or microwave oven, and stir well until dissolved. EZ-Block dissolves to give a clear solution. Use for blocking after the wash steps, and before incubation in any enzyme-conjugate solution (e.g. Streptavidin-HRP, Streptavidin-AP).

2. Optimization of Time Required for Blocking with EZ-Block

2.1. Cut 7 small squares of nitrocellulose or other suitable membrane.

2.2. Label each square with a ball point pen in 10 minute increments (60, 50, 40, 30, 20, 10), and one without blocking.

2.3. Place the first square (60) in a few ml of EZ-Block solution, and add successive squares at 10-minute intervals.

2.4. Wash all squares in TBST, PBST or Tris-Saline buffer (100mM Tris-Cl pH 7.5, 600mM NaCl).

2.5. Dilute the secondary antibody or streptavidin (HRP-conjugated) in EZ-Block solution.

2.6. Incubate on shaker for 1 hour.

2.7. Rinse in TBST, PBST or Tris-Saline buffer three times, 10 minutes each time.

2.8. Detect with ECL (EZ-ECL, Cat. No. 20-500-120).

2.9 Evaluate background intensity in each square. Select the incubation time that gives the lowest background.

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