Tribioscience

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Stripping Buffer (Catalog# TBS5020)

Description

Western Blot Stripping Buffer is a gentle method for breaking antibody-antigen interactions to allow nitrocellulose and PVDF membranes to be reprobed several times using different antibodies, saving time and conserving samples. Ideal for use with chemiluminescent substrates.

Directions for Use:

Used without dilution. One Western Blot Stripping Buffer package contains 500 ml Buffer A and 5 mL Buffer B, which combine to form sufficient reagent for approximately 25 membrane treatments.

• Warm bottle of Western Blot Stripping Buffer to room temperature.
• Wash already probed membrane with TBST (stock solutions available: TBS5008) for 5 min.
• Incubate the membrane on an orbital shaker with sufficient stripping buffer to cover the membrane. Depending on the size and quantity, more than 20 mL may be needed.
• Incubate/shake for 30 min at RT. Results can be had in as little as 15 min, with up to 2 hrs for strongly bound antibodies.
• Wash 3x with TBST for 5 min.

• If need, test for the removal of the immunodetection reagents as follow:

a. Test for complete removal of the HRP label (e.g., secondary antibody): Incubate the membrane with new ECL or Super Hi ECL Working Solution and expose to film. If no signal is detected using a 5-minute exposure, the HRP conjugate has been successfully removed from the antigen or primary antibody.
b. Test for complete removal of the primary antibody: Incubate the membrane with the HRP-labeled secondary antibody, followed by a wash in wash buffer. Incubate in new ECL or Super Hi ECL Working Solution and expose to film. If no signal is detected with a 5-minute exposure, the primary antibody has been successfully removed from the antigen.
c. If signal is detected, return to stripping step for an additional 15 minutes. Some antigen/antibody systems require increased temperature and/or longer incubation times to strip them fully. Optimize stripping time and temperature to ensure complete removal of antibodies while preventing damage to the antigen.
d. After determining that the membrane is properly stripped, the second immunoprobing experiment may be performed.

• Reblock with appropriate blocking reagent, and perform western blot onto membrane according to standard protocols.

Storage: Supplied as a ready to use solution, 500 mL. Store at 4℃.

RELATED PRODUCTS:

TBST-10x (catalog# TBS5008)
BSA Standard solution (catalog# TBS5002)
Protein Assay kit (catalog# TBS2005)
Cell Lysis Buffer(10x) (catalog# TBS5001)
SDS-PAGE running buffer (10x) (catalog# TBS5015)
Protein Loading buffer (6x) (catalog# TBS5014)
Protein Sample buffer (2x) (catalog# TBS5019)

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