

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 reprobbed 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 immunoprobng 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°C.

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)