Store at RT

Western Blot Stripping Buffer (5X)

#91925

100 ml

Support: +1-978-867-2388 (U.S.)
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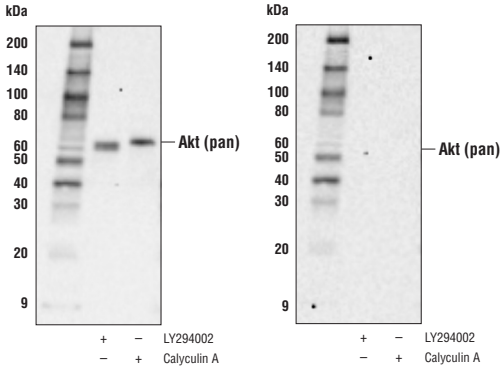
New 06/21

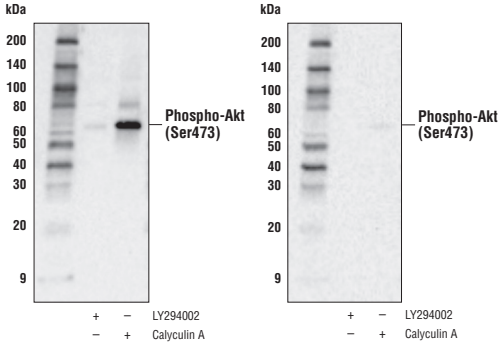
For Research Use Only. Not For Use In Diagnostic Procedures.

Description: Western Blot Stripping Buffer (5X) allows for quick and effective removal of both primary and secondary antibodies from nitrocellulose membranes without damaging the immobilized antigen, enabling the membranes to be reprobed with alternative antibodies or concentrations for further western blot analysis. This product should be diluted to 1X using RODI-water, and 2-mercaptoethanol should be added right before use. Completely submerge the membrane in Western Blot Stripping Buffer (1X). Optimization of incubation time or temperature may be required to achieve desired results.

Background: Having to re-run gels and repeat western blotting efforts is costly and time-consuming. Western Blot Stripping Buffer (5X) enables the membranes to be reused with the added benefit of not having to re-load expensive or limited samples into the gel. Allowing for the detection of a different target with an alternative primary antibody or by reprobing with a different concentration of a primary antibody saves valuable time.



Western blot analysis of extracts from serum-starved Jurkat cells, treated with LY294002 #9901 or Calyculin A #9902, using Akt (pan) (C67E7) Rabbit mAb #4691 before (left) and after (right) incubating the blot in Western Blot Stripping Buffer (1X) and then reprobing with secondary antibodies to show complete stripping of the membranes.



Western blot analysis of extracts from serum-starved Jurkat cells, treated with LY294002 #9901 or Calyculin A #9902, using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060 before (left) and after (right) incubating the blot in Western Blot Stripping Buffer (1X) and then reprobing with secondary antibodies to show complete stripping of the membranes.

Storage: Store Western Blot Stripping Buffer (5X) at room temperature. This product is stable for at least 12 months.

Thank you for your recent purchase. If you would like to provide a review visit cellsignal.com/comments.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Stripping Buffer Protocol

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

Supplied Reagents:

1. **Stripping Buffer (#91925):** To prepare 20mL of 1X Stripping Buffer, combine 4mL 5X Stripping buffer with 140uL 2-mercaptoethanol (0.1M final concentration) and 15.86mL RODI water.

Additional Reagents (Not Supplied):

1. **10X Tris Buffered Saline with Tween® 20 (TBST) (#9997):** To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH2O, mix.
2. 2-mercaptoethanol

B. Stripping Membranes

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

NOTE: This protocol begins after membrane exposure to film or digital imager. Best results are obtained if the membrane is not allowed to dry. Membranes can be stored in 1X TBST at 4°C.

NOTE: Quantitative comparison of targets before and after stripping is *not* recommended due to the removal of small amounts of membrane-bound protein with each round of stripping.

1. Wash three times for 5 min each with 15 ml of 1X TBST at room temperature.
2. Incubate membrane in 20 ml of Stripping Buffer for 45 minutes to 1 hr at 50°C with slight agitation, if available. Depending on antibody signal strength, optimization of incubation time and temperature may be required. The incubation time can be adjusted up to 2 hours while the incubation temperature can be adjusted to 70°C if needed.
3. Wash five times for 5 min each with 15 ml of TBST at room temperature.
4. (Optional) To check the efficiency of the stripping and confirm that the original signal has been removed, incubate the membrane with the appropriate secondary antibody and proceed with protein detection using recommended detection reagents. If stripping is complete, continue by washing the membrane five times for 5 min each with 15 ml of TBST at room temperature. If stripping is insufficient, repeat steps 2 through 4.
5. The membrane is ready for reuse. Proceed with the blocking step before adding the primary antibody.