

Stripping Western Blots

β -ME Stripping Buffer

62.5mM Tris-Cl pH 6.8 @RT
2% SDS
100mM β -mercaptoethanol

Make 125mM Tris-Cl (6.8), 4% SDS stock solution (2X)
Add β -ME fresh (143X)

For 20mL stripping buffer

10mL 125mM Tris/4% SDS
9.846mL H₂O
154 μ L β -ME

Prepare the stripping solution in a hybridization tube in the fume hood. Place membrane in hybridization tube, protein side should be facing the center of the tube. Heat to 50-65°C in the hybridization oven for 30min while rotating.

Remove the membrane from the tube and wash membrane in a sealed container with large volumes of TBST, approximately 1L TBST per membrane (5X 200mL washes).

There is a waste container for the stripping solution, and the washes can go down the sink.

Develop membrane to ensure stripping. Begin blocking...

Glycine Stripping Buffer

Make 2M Glycine HCl (pH 2.3) stock solution.

For stripping, dilute solution to 0.2M Glycine HCl and incubate membrane for 30min at RT while shaking on orbital shaker. Change buffer and repeat until membrane is stripped.

Wash membrane in TBST.

Collect the glycine buffer and pH to 7.0 with 10N NaOH. Dispose of the buffer in the sink.

Develop membrane to ensure stripping. Begin blocking...

The county of San Diego prohibits the pouring of liquids down the sink that are less than pH 5 and greater than pH 12.5