Stripping Western Blot

Solutions and Reagent

Note: Prepare solutions with Milli-Q or equivalently purified water.

Wash Buffer: 1X Tris buffered saline, 0.1% Tween-20 (TBS/T)

Stripping Buffer: To prepare 1 liter, mix 7.6 g Tris base, 20 g SDS, 7.0 ml 2-mercaptoethanol. Bring to 1 liter with dH_2O. Adjust pH to 6.8 with HCl.

Protocol

1. After film exposure, wash membrane four times for 5 minutes each in TBS/T. Best results are obtained if the membrane is not allowed to dry.

2. Incubate membrane for 30 minutes at 50°C in stripping buffer with slight agitation.

3. Wash membrane six times for 5 minutes each in TBS/T.

4. (Optimal!) To assure that the original signal is removed, wash membrane twice for 5 minutes each with 10 ml of TBS/T. Incubate membrane with ECL with gentle agitation for 1 minute at room temperature. Drain membrane of excess developing solution. Do not let dry. Wrap in plastic wrap and expose to x-ray film.

5. Wash membrane again four times for 5 minutes each in TBS/T.

6. The membrane is now ready to reuse. Start detection at "Blocking and Antibody Incubations" step in the Western blotting Protocol.