Chemiluminescent Detection with Pierce SuperSignal West Pico Substrate (34077)

2h blotting at 70V 6/17/05 new

1. Block membrane in 5% milk in Tris-saline pH 7.4 for one hour. (If high background is a problem, block with 1% BSA in Tris-saline.)
2. Wash 5 times in Tris-Saline. (If blocking with BSA, only wash twice.)
3. Incubate in primary antibody overnight.
4. Remove primary and wash blot 5 times in Tris-saline containing 0.05% Tween-20 (1:200 dilution of 10% Tween-20 stock). Optimally, each wash should last about 5 minutes, with agitation. TST
5. Incubate in secondary antibody, 1:50,000 dilution, in Tris-saline containing 1% BSA and 0.05% Tween-20 for 90 minutes. (Example: To prepare 10mls, combine 0.1g BSA, 50ul 10% Tween-20, and 10mls TS. Add 0.2ul HRP-conjugate directly into the ziploc bag.)
6. Remove secondary, wash blot 5 times in TS/0.05% Tween-20. Blot MUST be thoroughly washed after incubation with the HRP-conjugate. Increasing the wash buffer volume and/or number of washes can help reduce background.
7. Chemiluminescent Working Solution. Prepare by mixing equal parts of the Stable Peroxide Solution and the Luminol/Enhancer Solution. Pierce recommends using 0.125 ml Working Solution per square cm of membrane. Practically, you only need enough to cover the blot. **8 mls Working Solution (4mls each component) will cover a 13 x 15 cm blot**. Working Solution is stable for 8 hours.
8. Remove excess moisture from blot by laying it (protein side up) on a flat Kimwipe. Place blot in a glass tray or petri dish. Pour the Working Solution over the membrane. If done carefully, surface tension will hold the solution at the edges of the blot. Incubate for 5 minutes without shaking.
9. Pour off Working Solution, and place blot on a kimwipe to remove excess liquid. Place membrane in plastic wrap, pressing out any bubbles.
10. Expose to x-ray film in film cassette for 30 seconds and develop. Longer exposures can be done as needed. Light emission is most intense during the first 5-30 minutes after substrate incubation.