ECL Western Blotting Substrate

INSTRUCTIONS FOR USE OF PRODUCTS W1001 AND W1015.

Quick PROTOCOL

Protocol

Materials to be Supplied by the User

- blotted membrane
- blocking buffer
- wash buffer
- primary antibody
- HRP-conjugated secondary antibody
- tray for incubating and washing membrane
- rotary or rocking platform shaker
- X-ray cassette and film

1. After protein transfer, remove membrane from the transfer apparatus, and block nonspecific sites with either Tris-buffered saline (TBS), 0.05–0.1% Tween® 20, 2–5% bovine serum albumin (BSA); or phosphate-buffered saline (PBS), 0.05–0.1% Tween® 20, 2–5% BSA. Incubate for 1 hour at room temperature with shaking or 4°C overnight without shaking.

   Note: Milk may be substituted for BSA, depending on the primary antibody used.
2. Remove blocking solution, and add diluted primary antibody solution. Incubate for 1 hour at room temperature with shaking.
3. Wash 3 times, five minutes each wash, using TBS and 0.05–0.1% Tween® 20 (TBST).
4. Incubate membrane with diluted secondary antibody solution (conjugated to HRP) for 1 hour at room temperature with shaking.
5. Wash three times with TBST, 5 minutes each wash. Additional washes may help minimize background.
6. Prepare the substrate working solution by mixing equal parts of the Peroxide Solution and the Luminol Enhancer Solution. Mix just enough substrate to cover the membrane (e.g., 6–7ml per 10cm × 5cm membrane).

   Note: For best results, use the prepared substrate working solution immediately after mixing. The solution is stable for up to 1 hour at room temperature.
7. Incubate the membrane for 1 minute at room temperature.
8. Remove the membrane from solution, blot excess liquid with an absorbent towel, and place in a plastic sheet protector or clear plastic wrap.
9. Working in a dark room with a safe light, place covered membrane in a film cassette with protein side facing up. Place X-ray film on top of membrane, and expose for 1 minute. Exposure time can be increased or decreased to achieve optimal results, with light emission being most intense immediately after substrate incubation and significantly decreasing within 1 hour.

See additional protocol information in the ECL Western Blotting Substrate Technical Manual #TM317, available online at: ***www.promega.com/tbs/***

ORDERING/TECHNICAL INFORMATION:
www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

Promega

Printed in USA 6/09
Part# 9FB102