Alternative Western Blotting Protocol

(Produces less background than 4% BSA blocking)

1) Transfer gel to immobilization P membrane.

2) Block for 1-3 hrs in:
   2 ml 10X Western buffer
   16 ml dH_20
   2 ml Horse Serum
   Filter sterilize or add 0.2% NaN3 and save.

3) Incubate with primary antibody (1:500 dilution) overnight at 4°C in:
   4 ml 10X Western buffer
   28 ml dH_20
   8 ml Horse Serum
   Filter sterilize or add 0.2% NaN3 and save.

4) Wash (2 rinses, 1x15', 2x10') in:
   20 ml 10X Western buffer
   175 ml dH_20
   5 ml 20% Tween 20

5) Incubate with secondary antibody (1:10,000) for 40-60min at r.t. in:
   4 ml 10X Western buffer
   28 ml dH_20
   8 ml Horse Serum

6) Wash (2 rinses, 1x15', 2x10') in:
   24 ml 10X Western buffer
   84 ml dH_20
   12 ml 5% Tween 20

7) Develop blot using Amersham ECL reagents as described by manufacturer.

<u>10X Western buffer</u> (1 L)
1.5 M NaCl (87.66g)
150 mM Tris pH 7.4 (150 ml of 1 M or 18.2g)