# Western Blotting using Chemiluminescence

*Fabiola Paula Lopes[1], Alice de Moraes[1] and Peter J. Hansen[1]*

*[1]Depart. of Animal Sciences, University of Florida*

**Reagents**
ECL Western blotting kit (Amersham Life Science; cat# RPN2108): contains second antibodies for both mouse and rabbit, substrate and milk blocker (the milk blocker is not normally used when using ruminant samples).

Hybond ECL nitrocellulose membrane (Amersham Life Science; cat # RPN2020D)

Kodak X-OMAT (XAR-5, 18X24 cm; cat # 8532665)

**10X TBS**

12.11 g Tris-base (100 mM)

87.66 g NaCl (1500 mM)

1 liter dd $H_2O$

Adjust pH= 7.6

**Washing buffer (TBS-T):** 100 ml 10X TBS + 900 ml dd $H_2O$ + 1 ml Tween-20.

**Blocking buffer:** TBS-T + 1.5% gelatin

**Incubation buffer I (for first antibody):** TBS + 1.5% gelatin

**Incubation buffer II (for second antibody)** = blocking buffer (i.e., TBS-T + 1.5% gelatin)

## Procedures

1. Immediately after removal from the blotting apparatus, place membranes into blocking buffer for 2 hours. A small plastic gel box is a suitable container. This and all other incubation steps are performed at room temperature and in the rocker platform.
2. Wash membrane in washing buffer: rinse 2 times very briefly, incubate for 15 minutes, then repeat 2 x at 5 minutes each. Use a lot of buffer.
3. Transfer the membrane to a lid of 96-well microtiter plate or similar low volume container. Incubate with first antibody using recommenced dilution in TBS + 1.5% gelatin during 2 hours. Approximately 10 ml of diluted antibody showed to be sufficient.
4. Transfer the membrane into gel box and repeat step # 2.
5. Transfer the membrane back to the small container and incubate with anti-mouse or anti-rabbit IgG horseadish peroxidase diluted 1:8000 in TBS-T + 1.5% gelatin for 1 hour.
6. Repeat step # 4 (wash)
7. Prepare ECL solution for detection: mix equal volume of ECL reagent 1 and 2, (1:1) with final volume regarding of 0.125 ml/$cm^2$ (for a mini gel- 4 ml of each reagent).
8. Remove excess buffer from the membrane by draining the membrane over a piece of folded kimwipe paper and briefly touching the edge of the membrane to the paper.

9. Add ECL solution and incubate for 1 minute.
10. Repeat step # 8 ( drain excess of substrate)
11. Place membrane down on seran wrap, remove bubbles and wrap membrane completely.
12. Avoid excess amounts of seran wrap.
13. Tape membrane to the inside film cassette.
14. In the dark, add 1 sheet of x-ray film to the cassette. Expose membrane to film. It will probably be necessary to do several different exposures to find out the best exposure.
15. Develop the film.

*Blotting.wpd*

*1/30/97 converted to html 7-15-99, modified 10-21-99*