Western Blot Procedure

1) Scrape cells* off culture dishes and centrifuge.
2) Dissolve cell pellet in decanoyl-N-methyl glucamide (MEGA-10)** and clarify by centrifugation.
3) Mix 30 mg of protein*** with sample buffer containing mercaptoethanol and SDS and run on a 10% SDS gel. The protein was electroblotted on to nitrocellulose.
4) Block nitrocellulose with 5% powdered milk in PBS for 1 hour.
5) Wash the blot with PBS.
6) Add the antibody at a concentration of 1:1000 in 5% powdered milk/PBS and incubate for 1 hour.
7) Wash 3 x 5 minutes with PBS.
8) Add peroxidase-labelled anti-rabbit second antibody in PBS at a concentration of 1:3000 and shake for 1 hour.
9) Wash extensively with PBS.
10) Develop with ECL reagents (Amersham). For this experiment, the film was exposed to the blot for 10 seconds.

i. The cells used were the H4IIIE rat hepatoma cell line and the NBL-1 bovine renal epithelial cell line.
ii. The detergent used is not critical. MEGA-10 has the advantage of not interfering with the Bradford protein reagent.
iii. Whole cells were used in this experiment. If 30mg of a cell membrane fraction were used a more intense band would be seen.
iv. In this experiment, the antibody was used at 1:1000, but since whole cell protein was used and only 10 seconds development was required it could presumably be used at a lower concentration for many applications.