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NB600-101 Protocol

Western Blot protocol specific for Calreticulin Antibody (NB600-101)

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.
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i. The cells used were the H4IIE 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.