Semi-Quantitative Measurement of Proteins by Dot Blotting

(by Jun Takagi, 6/15/2000)

Purpose...

Concentration of proteins in a crude preparations (such as culture sup) can be estimated semi-quantitatively by using "Dot Blot" method if you have both purified protein and specific antibody against it.

Materials

TBS: 20mM Tris-HCl, 150mM NaCl, pH 7.5
TBS-T: 0.05% Tween 20 in TBS
BSA/TBS-T: 0.1% BSA in TBS-T
Nitrocellulose membrane (BIO-RAD, Trans-Blot™ etc)

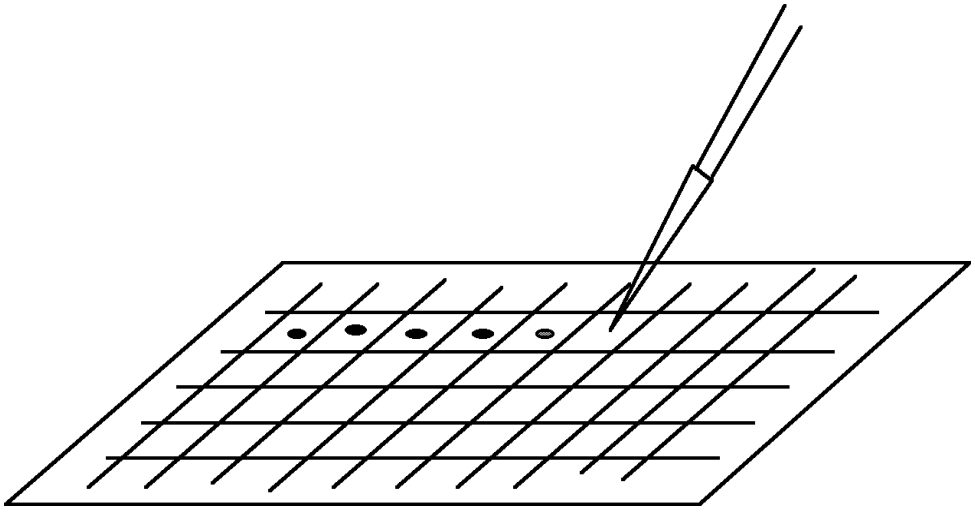
Procedure

1. Have nitrocellulose membrane (NOT PVDF!) ready, draw grid by pencil to indicate the region you are going to blot (see below).

2. Make serial dilutions of purified protein. For quantification of IgG, prepare the following solutions 0, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 ng/μl of mouse IgG in TBS

3. Make serial dilutions of your unknown sample, such as x1, x3, x10, and x30 dilutions for hybridoma culture sup. Make control dilutions (fresh culture medium at the same dilutions) as well.

4. Using narrow-mouth pipet tip, spot 1μl of samples onto the nitrocellulose membrane at the center of the grid. Minimize the area that the solution penetrate (usually 3-4mm diam.) by applying it little by little over 2-3 times of stroke of pipet.



5. Let the membrane dry.

6. Block nonspecific sites by soaking in 5%BSA in TBS-T (0.5-1h, RT). Use 10cm Petri Dish for reaction chamber.

7. Incubate with primary antibody (0.1-10μg/ml for purified Ab, 1/1000 to 1/100000 dil for antisera, x1 to x1/10 for hybridoma supernatant) dissolved in BSA/TBS-T for 30min at RT.

8. Wash three times with TBS-T (5min x 3)

9. Incubate with secondary antibody conjugated with HRP (for optimum dilution, follow the manufacturer's recommendation) for 30min at RT.

10. Wash three times with TBS-T (15min x 1, 5min x 2), then once with TBS (5min).

11. Incubate with ECL reagent (1:1 mixture of Solution 1 and 2, or you can dilute the mixed reagent 2 to 10-
fold with water if the signal was too strong) for 1min, cover with Saran-wrap (remove excessive solution from the surface), and expose X-ray film in the dark room. Try several different length of exposure.

12. Compare the signal from your unknown sample to that of standard and estimate the concentration.