

A technique for detecting, analyzing and identifying proteins, similar to the western blot technique but differing in that protein samples are not separated electrophoretically but are spotted through circular templates directly onto the membrane or paper substrate.

Concentration of proteins in crude preparations (such as culture supernatant) can be estimated semiquantitatively by using the dot blot method if you have both purified protein and a specific antibody against it.

## Reagents

### **TBS:**

20 mM Tris-HCl  
150 mM 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 the nitrocellulose membrane ready. Draw a grid by pencil to indicate the region you are going to blot.
2. Using a narrow-mouth pipette tip, spot 2  $\mu$ l of sample onto the nitrocellulose membrane at the center of the grid. Minimize the area that the solution penetrates (usually 2–4 mm diameter) by applying it slowly.
3. Let the membrane dry.
4. Block non-specific sites by soaking in 5% BSA in TBS-T in a 10 cm Petri dish (30 min to 1 h at room temperature).
5. Incubate with primary antibody (0.1–10  $\mu$ g/ml for purified antibody, 1:1000–1:100,000 for antisera, 1:100–1:10,000 for hybridoma supernatant) in BSA/TBS-T for 30 min at room temperature.
6. Wash three times with TBS-T (3 x 5 min).
7. Incubate with secondary antibody conjugated with HRP for 30 min at room temperature. For optimum dilution, follow the manufacturer's recommendation.
8. Wash three times with TBS-T (1 x 15 min and 2 x 5 min), then once with TBS (5 min).
9. Incubate with ECL reagent for 1 min, cover with Saran wrap (remove excessive solution from the surface), and expose X-ray film in the dark room. Try several different lengths of exposure.
10. Compare the signal from your unknown sample to that of the standard and estimate the concentration.