NovaTeinBio

Dot Blot

The following protocol is a simplified alternative method, the Dot Blot, to traditional Western blotting for the detection of the presence or absence of a particular protein or bio-molecule in samples. Dot Blot differs from Westerns in that proteins in the samples are not resolved by size prior to blotting. This method is better suited for comparing relative abundance of a target protein in a number of samples in parallel (low-, medium-, high-throughput).

Procedures

1. Label the nitrocellulose blotting membrane for the ease to locate your individual sample after blotting. For example, for 96-well sample dot blots, designate one corner of the membrane as well number A1, another corner as H12. Use indelible marker that will not bleed during processing.

2. Place the labeled membrane on a filter paper. Secure the edges of the membrane with tape or a weight to prevent the edge curling.

3. Pipette 0.5-2.0uL of each sample onto separate pre-determined locations on the blot. A multi-channel pipette/96 or 384-pin head may be used to spot multiple samples at once. The samples should be absorbed to the membrane immediately without any beading on the surface.

4. Block the membrane in 5% milk, 1x TBST for 30 minutes to 1hr on a rotating shaker.

5. While blocking, dilute the primary antibody in 5% milk, 1x TBST. Prepare enough diluted antibody to cover the blot during shaking incubation.

6. Decant blocking solution, rinse the membrane with 1x TBST, wash the membrane 2 – 3 times with 1x TBST buffer, each time 5 – 8 minutes with shaking on a rotating shaker.

7. Incubate the membrane with the diluted primary antibody for 30 minutes to 1hr on a rotating shaker.

8. If no secondary antibody or additional probes are necessary, proceed directly to step 10. Otherwise, discard the diluted primary antibody and wash with 5% milk, 1x TBST for 5 – 8 mins on a rotating shaker. Repeat this wash step 2 additional times for a total of 3 washes.

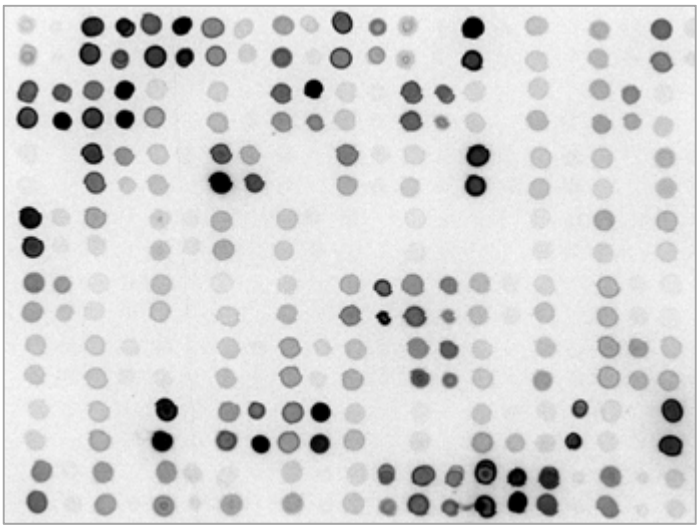
9. While washing, dilute the secondary antibody in 5% milk, 1x TBST. Prepare enough diluted antibody to cover the blot during shaking incubation.

10. After washing, incubate the membrane with the diluted secondary antibody. Incubate for 30 minutes to 1hr on a rotating shaker.

11. Discard the diluted primary/secondary antibody and wash with 1x TBST for 3 times, each time 5 - 8 minutes on a rotating shaker.

12. Proceed with the appropriate ECL substrate incubation and chemiluminescence/fluorescence detection as specified by the reagents manufacturer.

A sample Dot-Blot (spotted by 384-pin head), with 0.5 ul/spot:



Reagents and Buffers

1x TBST Buffer (1L)

100mL 10x Tris-Buffered Saline (500mM Tris pH 7.4, 1.5M NaCl)
10mL 10% Tween 20
890mL Deionized Water

1x TBST Buffer + 5% Milk (1L)

Note: Powdered non-fat dry milk must be thoroughly dissolved into buffer before Tween-20 addition so that no sediment remains.

100mL 10x Tris-Buffered Saline (500mM Tris pH 7.4, 1.5M NaCl)
10mL 10% Tween 20
50g Blotting Grade Non-fat Dry Milk
890mL Deionized Water