

Dot Blot Protocol

Buffers and Reagents:

Dilution buffer: PBS with 0.5 % glycerol and 0.006% Pyronin Y - Antigens should be diluted in Dilution buffer to spot them on membrane.

Membrane: PVDF membrane such as Trans-Blot Transfer Pure Nitrocellulose Membrane

Blocking solution: Blocking agents that 1% BSA, eg. <http://www.fitzgerald-fii.com/bsa-reagent-grade-30-ab81.html> , Non-fat dry milk, serum are commonly used.

1% BSA/PBS-T or 0.25% Skimmed milk/PBS-T are usually used (TBS may also be used instead of PBS).

Wash solution: PBS-T (or TBS-T) PBS or Tris -buffered saline (pH 7.4) with 0.05% (v/v) Tween 20

PBS <http://www.fitzgerald-fii.com/pbs-10x-concentrate-85r-125.html>

Secondary antibody: See our full list of conjugated secondary antibodies at <http://www.fitzgerald-fii.com/secondary-antibodies.html>, and choose by source animal, conjugated substance for your choice.

HRP Luminol Substrate

Some other useful reagents: <http://www.fitzgerald-fii.com/diluents.html>

General Procedure:

Blotting membrane

1. Dilute the antigen to a final concentration of 10-50 µg/ml in Dilution buffer.
2. Grid the membrane using pencil (or other tool) to identify the position of antigen spotting.
3. For hydrophilic treatment, treat membrane with methanol and washed with TBS-T.
4. Spot the antigen on membrane.
5. Let the membrane air-dry.

Blocking

6. Soak the membrane in blocking solution over 30 minutes at room temperature.
7. Wash three times with TBS-T (3x5 minutes)

Primary antibody

8. The primary antibody is diluted to an optimized concentration in blocking buffer.
9. Incubate the membrane with primary antibody solution for 1 hour at room temperature.
10. Wash the membrane three times with TBS-T (3x5 minutes)

Secondary antibody

11. The Secondary antibody is diluted to an optimal concentration in blocking buffer immediately before use.
12. Incubate the membrane with secondary antibody solution for 30 minutes at room temperature.
13. Wash three times with TBST (3×5 minutes).

Detection

14. Incubate the membrane with HRP luminol substance about 30 sec to 60 sec and remove excessive solution from membrane.
15. Observe membrane (cover with saran-wrap) by lumino image analyzer.