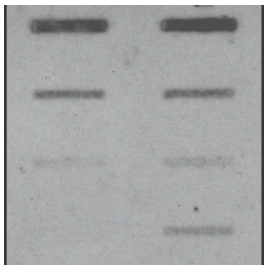
Sep 08, 2020

Slot/Dot Blot protocol.



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Protocol status: Working
We use this protocol in our lab and it is a great time saver.

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Keywords: dot blot protocol, detecting antigen, antibody, protocol, secondary antibody incubation, antigen, secondary antibody, simple slot, slot

Disclaimer

Abstract

This is a simple slot/dot blot protocol for detecting antigen on a membrane. Using the method outline here, one can achieve results in under 90 min because it eliminates the need for running a gel, transfering the protein in a transfer apparatus, blocking, secondary antibody incubation and the washes between primary and secondary antibodies.

Troubleshooting

Set up Slot/Dot blot apparatus

5m

1 5m

1. Assemble 3 sheets of cut GB003 blotting paper pre-wetted with transfer buffer (25 mM Tris, 200 mM Glycine, 20% methanol) to the dot blot apparatus.
2. Pre-wet a cut sheet of PVDF in 100% methanol for 30 sec and then wash the PDVF in transfer buffer for 2 min.
3. Lay the PVDF membrane on the GB003 blotting paper and seal the slot/Dot blot apparatus.

Add sample

5m

2 5m

1. Apply individual (or pooled) saliva or processed nasal swabs to the slot/dot blot apparatus making note of sample location.
2. Turn on the vacuum source so the sample get drawn onto the membrane.
3. Disassemble the slot/dot blot apparatus and place the membrane back in 100 % methanol.
4. Air dry the membrane for 5 minutes.

Incubate with HRP-conjugated antibody, wash and develop

1h

3 1h

1. Once the membrane is dry, add it to 10 ml of TBST (20 mM Tris, 150 mM NaCl, 0.1% Triton X-100, pH 7.5) (or PBST) containing HRP-conjuated antibody and incubate with gentle shaking 40 minutes.
2. Wash the blot 4 times for 5 minutes with 10 ml of TBST
3. Add ECL or colorimetric substrate, develop for 2-5 min, and image blot.
4. Record the results.